Binary Fission in *Collodictyon triciliatum* Carter

by

Robert Clinton Rhodes

A thesis presented to the Faculty of the

College of Letters and Science

in the

University of California

In partial fulfillment of the requirements

for the degree of

Doctof of Philosophy.

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A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
AT THE UNIVERSITY OF CALIFORNIA

BY

ROBERT CLINTON RHODES

1917

UNIVERSITY OF CALIFORNIA PUBLICATIONS
IN
ZOOLOGY

Vol. 19, No. 6, pp. 201-274, plates 7-14, 4 figures in text. December 3, 1919

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UNIVERSITY OF CALIFORNIA PRESS
BERKELEY

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Cited as Univ. Calif. Publ. Zool.

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CONTENTS

INTRODUCTORY AND HISTORICAL

In February, 1916, I found in an aquarium containing goldfish a dominant and persistent, free living flagellate which seemed to warrant further investigation. Being large and transparent, except when filled with green and opaque inclusions, and occurring in quantities, it presented an opportunity for continuous and repeated

observations, especially upon its metabolic character and nuclear changes. The form has been identified as *Collodictyon triciliatum* Carter (= *Tetramitus sulcatus* Stein).

This genus was first described by Carter (1865, p. 289) as follows:

> *Collodictyon*, nov. gen. *C. triciliatum*, nov. sp.
>
> Pyriform, straight, or slightly bent upon itself, bifid at the small extremity, presenting at the larger one an indentation, from which spring three cilia. Structure transparent, cancellated, composed of globular cells, with a strongly marked, greenish granule here and there in the triangular spaces between them. Locomotive, swimming by means of the cilia; subpolymorphic, flexible, yielding, capable of assuming a globular form . . . or one more or less modified by the body it may incept . . . ; enclosing crude material for nourishment in stomachal spaces, and ejecting the refuse, like *Amoeba*. Provided with a nucleus and contracting vesicles.

He gave its habitat (p. 289) as "fresh water, chiefly among *Euglena* and Infusoria of that kind." Its length was 1/771 in. (30μ) and its location the Island of Bombay. Among his observations he added (p. 289) the following: "The plastic nature of this Infusorium, and its mode of incepting food being like that of *Amoeba* (for it does not appear to possess any oral aperture), induce me to think that it should be placed among the Rhizopoda. Still it seems to have some analogies to *Bodo* Ehr." "Its generic name has been derived from its plasticity and delicate cellular structure, which gives it a reticular or cancellated appearance; and its specific designation from the presence of three cilia."

The above description is satisfactory for identification, though not detailed. My own observations coincide with it with these exceptions: there are four instead of three flagella; it may or may not be bifid at the posterior end; there is at the anterior end of the body a blepharoplast from which the flagella arise, these not springing from, but near the indentation, which is a continuation of the median groove, or sulcus, which functions in food ingestion; I have found no contracting vacuole, though Carter stated that he had observed "contracting vesicles" having no fixed position, but he figures none.

In 1878 Stein figured a similar form, showing, however, the four flagella, a median sulcus and a contractile vacuole, naming it *Tetramitus sulcatus*. Kent (1880–1882, p. 314) accepted these organisms as described by Carter and Stein as belonging to separate genera of the family Trimastigidae, but that there is little cause for such a distinction can be seen from his characterization of Stein's genus as follows:

Body obtusely pyriform or subcordate, widest and rounded anteriorly, tapering towards and bluntly pointed at the posterior extremity, about one and a half times as long as broad; a deep groove traversing the entire length of the centre of the ventral side and imparting to the posterior extremity, as seen from beneath, a bilobate contour; flagella four in number, of equal length, inserted close together in the centre of the anterior border; endoplast and contractile vesicle located side by side near the same anterior margin; parenchyma granular, soft and plastic. Length 1/700. Hab., fresh water.

Bütschli (1887, p. 841) recognized these two forms as the same and *Tetramitus sulcatus* Stein as a synonym of *Collodictyon triciliatum* Carter, characterizing the genus as follows under the family Tetramitina:

Mässig gross (L. bis 0,035 Mm.), estalt vorn etwas verbreitert und quer abgestutzt, nach hinten wenig verschmälert und abgerundet. Wahrscheinlich etwas abgeplattet; über die eine Fläche zieht eine breite Längsfurche hinab. Vorderende mit vier gleich langen aus einem Punkt entspringenden Geisseln (Carter gibt nur drei an). Nucleus und contractile vacuole im Vorderende. Nahrungsaufname sicher. Vermehrung durch Längstheilung. Süsswasser. Europa und Ostindien.

In 1893 Klebs investigated a form which he designated as *Tetramitus sulcatus* Stein. He found the four flagella of unequal length and a contractile vacuole in the posterior end of the body, the longitudinal furrow a spiral, the size being 17 by 15 microns. These differences lead me to conclude, after careful consideration of his description and figures, that he is mistaken in his verification and the form he described is not *Collodictyon triciliatum,* but probably some species of *Tetramitus.*

Francé (1899) studied Carter's organism thoroughly; his description is accurate and detailed, his figures typical and true to life. My own observations coincide with his in practically all details. He concluded that Klebs' (1893) description was not of *Collodictyon.* He dealt fully with the morphological features and metabolic changes, to which I have little to add. In only one important point do our observations fail to agree. I can find no contracting vacuole. He left untouched, however, the method of mitosis, merely stating that reproduction was by longitudinal division, which was also noted by Carter (1865); it is to this especially that I shall address myself.

I am indebted to Dr. Olive Swezy for suggesting the desirability of working out the mitosis of this form, the correction of the bibliography, sketching figures 75, 78, and 83 of plate 8, in my absence, and for repeated criticism and help. I also wish to thank Professor

C. A. Kofoid for his suggestive interpretations and criticisms, both constructive and destructive, and for the determination of the extranuclear division center.

MATERIAL AND TECHNIQUE

Plentiful material was found in an aquarium where goldfish were kept. These were obtained from the Yorizuya Company, who represent the Nippon Gold Fish Company and import direct from Japan. In no other cultures have I found *Collodictyon*. Since it has only been noted from India and central Europe, it is possible that it is not native to California, and may have been introduced with the importation of goldfish from Asia or Hawaii. On the voyage from Japan, however, the barrels in which the fish are contained are emptied and fresh water added at Hawaii and also at the wharf in San Francisco. It would be easy for the flagellates to be brought through notwithstanding this change of water, either by being transferred with the fish, with water plants which are brought in the same aquaria, or by clinging to the moist sides of the containers (barrels). Thus, though there is a possibility that *Collodictyon* has been introduced into California, the cosmopolitan distribution of Protozoa makes this highly improbable, and this genus may be regarded as indigenous.

These forms have persisted and usually have been dominant in an aquarium $26.5 \times 60 \times 20$ cm., the bottom of which is covered with sand to a depth of about an inch, in which *Ulothrix* has grown in quantities and to which I have added *Lemna, Ranunculus,* and *Myriophyllum.* At least one goldfish has been present all the time, at times two and four. These fish have fed freely on the plant life of the aquarium, making it necessary to replenish all higher plants and on more than one occasion, the algae, although the aquarium has been sufficiently well balanced for one fish to survive since January, 1916. The aquarium has been placed outside a window with southern exposure, partly protected by glass plate and wooden cover. The variations of temperature for the year have been from about 28° F to 92° F. Considerable variation of temperature from the heat of the direct rays of the sun at mid-day to the cool nights failed to destroy the culture. Since the cooler weather of last December *Collodictyon* has been supplanted at intervals by a dinoflagellate, *Peridinium penardii* Lemm., as the dominant organism, the latter seeming to be favored by the cooler weather. On one occasion the aquarium froze over during the

night, the ice being one-eighth to one-quarter of an inch in thickness, forming at a temperature of 28° F. Under the ice and throughout the following day *Collodictyon* seemed even more numerous than ever and many dividing forms were found. The following night, temperature 32° F, abundant mitotic stages of *Collodictyon* and *Peridinium* were found. Many of the dinoflagellates escaped from their theca by ecdysis and many division stages were observed. Greeley (1903) noted the effect of reduction of temperature upon the artificial production of spore formation and multiple fission in *Monas*. I obtained nothing resembling resting spores or somatellas. At 4° C Greeley (1903) found that *Monas* rounded up into a resting spore in about six hours, and at 1° C multiple fission, resulting in a resting somatella, was observed within five days. It is interesting to note in comparison that binary fission was simply accelerated in both *Collodictyon* and *Peridinium* at a temperature of 32° and 28° F.

Collodictyon seemed most abundant on the surface, naturally tending to accumulate in the corners and around the edges of the aquarium. But during the day and night at all temperatures above freezing, I have found them present throughout the aquarium, from the bottom to the top, under the protected area as well as the open end. I placed slides and covers, suspended at various depths, as well as covers suspended in cylinders to eliminate currents, and found abundant organisms in all parts of the aquarium. In these experiments, attempted primarily to determine the time and conditions of division and probable multiple fission, I was led to conclude that division was determined more by chemical than physical conditions, that it was no more abundant at night than during the day, that individuals undergoing binary fission remained at the surface for the most part, but could be found at all depths, in all degrees of light and temperature, though more abundant at 32° F. At no time have I found a clear case of multiple fission. One instance (pl. 7, fig. 62) of a somatella was observed, but on careful comparison of the staining reactions, I was led to conclude that this was a cyst of *Amoeba radiosa*, vegetative stages of which were abundant in the aquarium. The life cycle of *Collodictyon*, as far as traced, is thus simple, reproduction being by binary fission only. When the organisms wholly disappear from the cultures, I have been unable, by varying conditions, to start the culture up again. It seems, therefore, that there may be no cysts or resting stages for this flagellate, at least under the conditions observed. The extreme variations in size (pl. 2, figs.

5–6) would naturally lead one to suspect a somatella stage, though this variation may be accomplished by reduction through successive binary fissions.

In culture experiments I have tried malted milk (one-sixteenth of one per cent solution and varying strengths), crushed *Myriophyllum*, boiled mushroom solution, amoeba agar, sterilized soil with tap water boiled thirty-five minutes, beef extracts, and quince-seed jelly as suggested by Turner (1917) for *Euglena*. Most of these were partially successful, but only temporarily so, *Collodictyon* soon disappearing from the culture.

Among associated forms in the aquarium, I have found: *Pandorina, Peridinium, Euglena, Amoeba* of the *limax* group and others, *Platydorina, Gonium, Actinophrys, Bodo, Chlamydomonas, Chilomonas, Coleps, Stylonychia, Euplotes patella* and *E. charon, Microthorax*, and *Colpidium;* rotifers (*Branchionus, Philodina,* and *Chaetonotus*); *Ulothrix, Oscillatoria, Chlorella, Tetraspora, Spongomonas, Lagerheimia, Scenedesmus, Pediastrum, Selenastrum, Coleochaete, Navicula, Closterium, Cosmarium,* and several undetermined desmids and diatoms.

I have considered the possibility that the life history of *Collodictyon* may in some way be related to its association with goldfish. Aside from the balancing of the plant and animal life of the aquarium, I have looked for some symbiotic or parasitic relationship, but have found none, other than the fact that I have been unable to keep a permanent culture in other than a goldfish aquarium. I tried a fairly well balanced stickleback aquarium without success. In aquaria where there were abundant *Ulothrix* and other algae, *Collodictyon* did not persist. The voracious habits of this animal led me to believe that it is not symbiotically dependent upon goldfish, nor has it any other method of food-taking involving absorption; but its habits of engulfing free living protozoa and algae may wed it to a well balanced condition such as would be found in a satisfactory goldfish aquarium. There is a bare possibility that the life-history is dependent upon the presence of living fish, though Carter and Francé's observations are opposed to such an interpretation. In examination of stomach and intestinal contents I found no evidence of symbiosis or parasitism relating the two. Examination of the gills and body for ectoparasites never failed to yield some of these forms. This was probably due, however, to their abundance in the water.

It is rather a prevalent custom to use cover-glass preparations in

protozoan technique. I refer here not to Romanowsky's dry film, which has no general use or approval, but to a modification of Schaudinn's moist film method, which involves the use of some fixative, pipetting the organisms on to the cover smeared with this and then the evaporation of the water until the animals will adhere to the cover when dropped or floated directly on the surface of the killing fluid. I found on attempting this method that conditions accompanying the evaporation process ruptured the body, and normal killing or fixation could not be obtained with *Collodictyon,* which is evidently more susceptible than many other free living forms.

Collodictyon when exposed to excessive evaporation may rupture instantaneously or more frequently pass through moribund phases as shown in text figure C. All foreign bodies are ejected, the vacuoles become larger, gradually fuse into still larger pathological vacuoles, which finally rupture. The organism becomes much distorted and disintegration usually takes place along the sulcus.

Wherever the death rate is above the normal or even at equilibrium, the number of abnormal moribund forms is undoubtedly great. Many life cycles could be and possibly have been built up on such fallacious interpretations. Protozoa may be potentially immortal but the vast majority do not survive. The real problem, therefore, is to find some way to determine the normal from the moribund, either physiologically or pathologically abnormal.

I resorted to killing *en masse,* either with or without centrifuging. I found that in lightly centrifuged material there was no nuclear displacement or other variations which could be detected from the non-centrifuged, so I adopted the centrifuge as the best and quickest method of running up the material. After a normal killing in a beaker and running up through the alcohols in centrifuge tubes, some of the organisms were then fixed to covers, where it was essential for the quick handling of material, as in Mallory's stain. I also found it convenient and satisfactory in dehydrating rapidly after certain stains, as in phosphotungstic haematoxylin, to pass from an aqueous stain by adding 3 to 5 c.c. of 50 per cent and then 40 to 50 c.c. of absolute alcohol, immediately centrifuging and adding carboxylol.

At times *Collodictyon* was found to be rapidly increasing in numbers in the aquarium and from this it was judged that there was little or no death taking place, but that conditions were favorable for a maximum growth and normal reproduction. Material collected at such times as this was regarded as normal, at least for the phase of

purely vegetative existence. By collecting water from the aquarium and putting in petri dishes I was at times able to get a determinate increase in numbers and low mortality. By killing at such times, material normal as it was possible to get was obtained. Various methods of introducing the material to the killing fluid was used: by pipette, by pouring, or by pouring the killing fluid on concentrated masses of the organisms. The proportion of the killing fluid was never allowed to be less than ten times the amount of the water containing the flagellates.

The most satisfactory material was obtained by killing either in hot Schaudinn's fluid or strong Flemming, and staining in Heidenhain's aqueous iron haematoxylin. By this method the nuclear differentiation becomes evident as figured in accompanying plates. Counterstaining in eosin, either just after destaining or from 50 per cent alcohol, brings out the flagella.

Other killing fluids used in addition to Schaudinn's and strong Flemming were: picro-mercuric, Gilson's, Flemming's weak, Carnoy's, Zenker's, Bouin's, and osmic acid. None of these was perfectly satisfactory, possibly through lack of proper proportions or some undetected flaw in technique.

Heidenhain's aqueous iron alum haematoxylin I regard as by far the safest, most differential and permanent stain. Alcoholic solutions were about as satisfactory, but required from one to two hours for the mordant and eight to twenty-four for the stain, thus not being much quicker than the aqueous solutions. Acid fuchsin or eosin were excellent counterstains with either of the above. Delafield's haematoxylin yielded beautiful but not so critical a differentiation. The granular organization of the chromatin, even of the karyosome, was emphasized. Phosphotungstic acid haematoxylin was of assistance in determining the nuclear membrane, but did not show up the spindle fibers as was hoped. Mallory's connective tissue stain as modified for Protozoa yielded only fairly satisfactory results with the fixatives tried. By its use the two basal granules embedded in the blepharoplast could be distinguished. For some reason *Collodictyon* seems able to withstand chemicals, especially the usually quick stains, far more than other free living Protozoa with which I have dealt, the time required for all stains being longer than that ordinarily scheduled. Flemming's safranin, gentian-violet, orange G was tried, for the purpose of differentiating the macrokaryosomes and microkaryosomes, but no variations of color diagnostic of chemical differences were obtained. The

cytoplasmic differentiation, however, was good. The blepharoplast and evidence of a rhizoplast were emphasized by preceding Heidenhain's iron haematoxylin with weak Bordeaux red for twenty-four to thirty-six hours, and destaining until the chromatin was almost colorless, as directed by Heidenhain. Paracarmine with aluminum chloride as mordant did not yield satisfactory results. *Collodictyon* proved susceptible to neutral red and methylen blue of the intra-vitam stains. It was unaffected by Bismarck brown. Neutral red after a considerable time differentiated the food vacuoles and acted somewhat upon the plasma. It never disclosed a pulsating vacuole. Methylen blue largely reacted upon the plasma and was not differential, possibly showing up the protoplasmic vacuoles with some intensity.

In order to section I resorted to the capsule method of embedding. The material was taken from the xylol-paraffine, saturated solution, in the centrifuge tube, and placed in a capsule. Melted paraffine was dropped in and this process was repeated successively until the material was satisfactorily embedded. Sections were cut 3, 5, and 7μ thick.

GENERAL MORPHOLOGY

Collodictyon is variable both in size and shape. Its length is 15 to 60μ; width, 10 to 40μ; thickness, 8 to 36μ. These figures show that many of the individuals I have found are smaller than those recognized by previous observers. The typical shape of the body (pl. 7, figs. 1–4) is ovoid, cordate or bifurcated posteriorly. A longitudinal furrow, or sulcus, is always present, though at times showing only a slight indentation, but usually evident as a deep groove on one of the narrower sides. Four equal flagella about as long as the organism arise from the anterior ovoid end. Anteriorly the body is ovoid, at times cordate, the sulcus extending around as an indentation. The general shape is rounded or compressed in the plane running through the sulcus, the nucleus and the blepharoplast. The posterior end may be truncated, oval, acuminate, bifid, with the cusps pointing posteriorly, curved spirally or diverging at an angle up to seventy-five degrees, or with three, four, or five cusps (pl. 7, figs. 5–8) caused by secondary sulci which run parallel to the primary longitudinal sulcus. Changes of form are gradual except when altered by engulfed food or the extrusion of undigested products. The peculiarities of the posterior cusps are held by a single individual indefinitely. Few individuals, if any, are exactly alike in form.

Seldom does the sulcus extend far enough forward to modify the regularity of the anterior end, which is fairly constant in form, much more so than the posterior end. This sulcus is a furrow or depression which cleaves the body on one side, which side may consistently be called sulcal or adsulcal, analogous to ventral, as opposed to absulcal, which is analogous to dorsal. The secondary sulci usually branch from the chief longitudinal sulcus, the resulting cusps being variable in size, shape and permanence. At times the general form becomes spherical and globular, the posterior end truncated, or ovate and conical, with acute posterior end, the sulcus being faint in both cases.

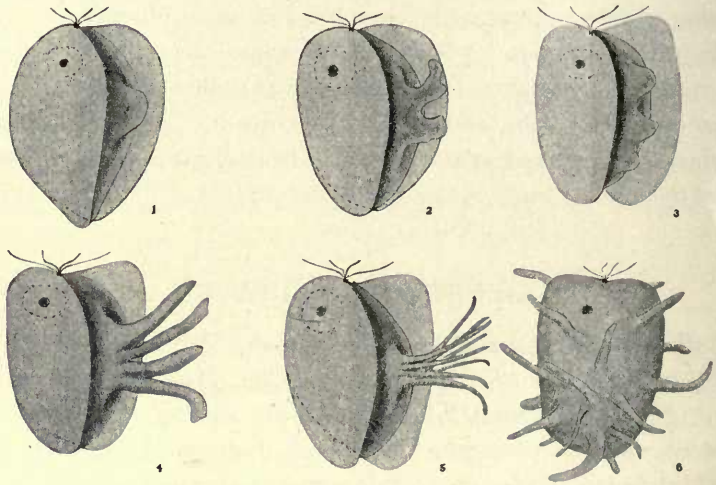

Fig. A. Pseudopodia of *Collodictyon*. Diagrammatic. × 1000. 1–2. Lobose. 3. Undulate. 4. Digitate. 5. Filose. 1–5. From the sulcus. 6. From all parts of the surface.

The four flagella are paired, each pair arising from a single basal granule, the two granules being embedded in the irregular chromatoidal blepharoplast which is surrounded by a granular, less darkly staining archoplasm or modified cytoplasm. The flagella are typically whip-like, in length averaging that of the major axis of the body, at times a little longer, measuring in one instance 68 to 70μ. They taper toward the tip. Francé (1899) used zinc chloride to bring out their full length and further observed their base to be granular. Language is inadequate to describe the beauty of their elegant backward curves. They function both in pulling and propelling the body forward, in attachment to the substrate while the organism rotates on its major axis, as a tactile organ for directing locomotion, and actively in offense

and defense. In attacking a dinoflagellate they were observed to catch their prey with all four flagella; they then allowed themselves to be pulled about until the dinoflagellate was exhausted, when it was drawn back to the anterior end of the sulcus and engulfed. On another occasion when *Collodictyon* was being drawn toward the mouth of a rotifer, it spread its flagella and lodged upon the oral membrane; at other times it was enabled to guide itself to one side of the oral current. While not the most conspicuous, the flagella constitute the most useful organelles of this slightly differentiated unicellular organism.

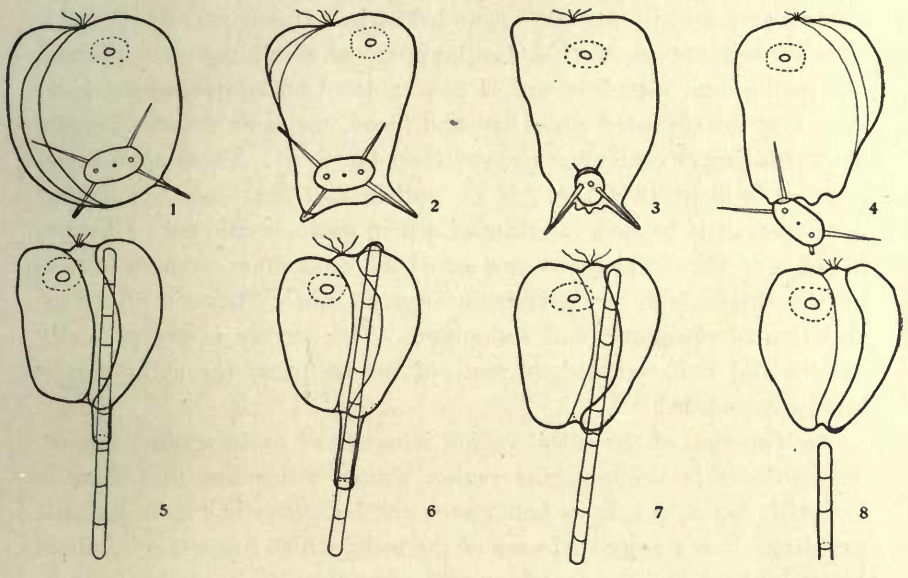

Fig. B. 1–4. Extrusion of *Lagerheimia* by *Collodictyon*, showing false pseudopodia. 5–8. Unsuccessful attempt of *Collodictyon* to engulf long *Ulothrix* filament. Diagrammatic. × 750. 5. Extension of protoplasmic sheath along filament. 6. Contraction of protoplasmic sheath forcing the filament anteriorly, pushing out the surface. This was repeated several times. 7. Retraction of protoplasmic sheath. 8. Extrusion of filament.

The sulcus is usually a smooth depression, but when *Collodictyon* is actively searching for food, pseudopodia are extended from any or all portions of the sulcal region. These may be of various types, hatchet-shaped, lobose, finger-like, or filose (text fig. A, 1–5). Finger-like pseudopodia from over the entire surface of the body were observed (text fig. A, 6), but such are very rare. The entire sulcus at times may become serrated and undulating, exceedingly amoeboid and metabolic. The posterior end may put forth lobose pseudopodia or flow around objects being engulfed (text fig. B, 1–6). The posterior cusps

are themselves slightly amoeboid and may slowly change position and form, though this is unusual.

On many occasions I have noticed a change of form of a different character and at first thought true pseudopodia were being extended from the whole body surface, but by continued observations it was discovered that some undigested plant, as *Scenedesmus* or *Lagerheimia,* was escaping or being extruded (text fig. B, 1–4). The pellicle and cytoplasm extended out around the rays or processes and undoubtedly part of the cytoplasm was lost when the object was set free. The cytoplasm immediately contracted, the ruptured edges or margins drew closer together until they were rounded up and fused (text fig. C, 1–7). This seemed not so much a healing process as simply protoplasmic contraction and rounding up. I have noticed on two occasions, however, that the ruptured edges met and fused, enclosing a water vacuole in the healing or coalescing process (text fig. C, 5). These phenomena make it evident that there is no cuticle and that the pellicle has developed little beyond the state of a firm protoplasmic gel. Changes in shape in the sulcal region are amoeboid, most other changes involving the whole body are metabolic or euglenoid. There is no differentiation of ectoplasm and endoplasm. The surface is exceptionally smooth and well rounded, in spite of the fact that the cytoplasm is highly vacuolated.

The function of the sulcal region is not hard to determine, for undoubtedly it is through this region almost altogether that food is ingested; but to decide its true status and homology is a more difficult problem. It is a restricted area of the body, which has either retained or evolved an amoeboid and miscible surface. It is always a unit, a persistent though variable character, and of constant function. Amoeboid pseudopodia are almost entirely restricted to this area. Many flagellates, such as *Euglena* and *Astasia,* are exceedingly metabolic, constantly varying in shape, but they have no such differentiated surface area as this found in *Collodictyon. Mastigamoeba* is classified as a polarized rhizopod, the entire surface of which is amoeboid. The exceeding voraciousness of *Collodictyon* is indicative of a fairly advanced organism and in view of this fact it seems best to regard the whole sulcal region as a modified cytostome. It must be considered more homologous to the amoeboid surface of *Mastigamoeba,* however, than to the more restricted gullet of *Euglena,* which is probably homologous with only the anterior end of the sulcus. This structure indicates a possible origin of the more specialized cytostomes as found

in *Trichomonas, Costia,* and *Giardia. Collodictyon* must in any case be looked upon as a form of simpler organization and probably of a more primitive type than these parasitic forms.

The anterior extremity of the sulcus, just beside the base of the flagella, may be modified into a depression (pl. 9, fig. 19) which has

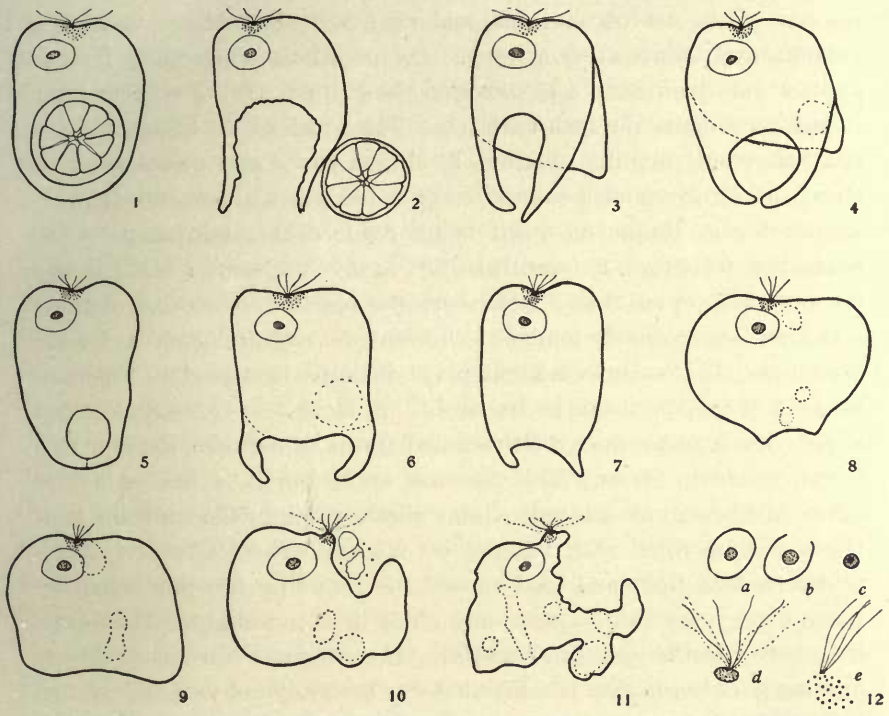

Fig. C. Escape of *Pandorina* and subsequent dissolution of *Collodictyon* due to drying. Diagrammatic. × 750. 1. *Pandorina* within food vacuole. 2. *Pandorina* swimming away; ruptured surface of *Collodictyon.* 3, 4. Apparent healing of torn surface. 5. Fusion of ends of protoplasmic processes, enclosing water vacuole; of frequent occurrence. 6–8. Resorption of protoplasmic processes. 8, 9. Flattening of body; formation of pathological vacuoles indicative of dissolution. 10. Bursting of vacuole at anterior region of sulcus. 11. Further dissolution, rupturing posteriad along the sulcus. 12. Nucleus and blepharoplast freed by dissolution. *a.* Nucleus; nuclear membrane persisted for two minutes. *b.* Rupture of nuclear membrane. *c.* Karyosome; persisted for thirty minutes; finally broke up into small granules. *d.* Blepharoplast; basal granules surrounded by archoplasm, flagella still moving. *e.* Rupturing of archoplasmic mass; flagella cease beating.

a probable function of a more specialized cytostome. It takes the form of a permanent depression in an amoeboid surface. It does not usually function in food getting, for this is accomplished by the amoeboid surface of the entire sulcal region. I have, however, observed unicellular algae and small dinoflagellates engulfed through or near this

depression. Here then is a structure which by location and analogy may be correlated, or at least compared, with the highly specialized gullet of forms like *Euglena*.

There is a tendency at times for *Collodictyon* to become exceedingly eccentric in its form (pl. 8, figs. 9–18). Its irregularity is for the most part accompanied by a flattening and warping, the sulcus cleaving one of the narrow margins, making a secondary if not, indeed, a fundamental bilateral symmetry. On occasions when such irregularities were prevalent, I have tested the culture, trying to determine if possible a cause for such variation. The water of the aquarium was neutral or only slightly alkaline, by litmus paper and litmus solution tests. Alkalinity tended to produce rounded, globular, conical, or pear-shaped forms, the sulcus itself being reduced to a minimum. Concentration tests were not accurate, but in my judgment I could detect no variation upon this. Death always resulted when the density was such as might be judged sufficient to rupture such a fragile organism. That oxygen content plays an important part in the variation in shape there can be no doubt. Sufficient or excessive oxygen supply tends to produce well rounded forms, a deficient supply, flattened, eccentric forms. This test was easily made by having a substrate of filamentous and unicellular algae, which in the sunshine kept the aquarium filled with bubbles of oxygen. The alternative interpretation that light and heat caused the rounding up, was tested by placing the aquarium without any algae in the sunlight. The forms then retained their original shapes. The chemical content so far as organic salts in solution is concerned was probably not variable enough to produce the variations, tests having been made for sodium, calcium, and magnesium salts with negative results. By adding carbon dioxide slowly in small quantities similar eccentricities of shape resulted as from deficient oxygen. From these tests I concluded that variation of shape was largely a question of respiration, irregularities being either degenerative stages or adaptations to meet deficient oxygen supply. Carbon dioxide in excessive amounts would be immediately converted into carbonic acid gas and thus make the culture slightly acid. This is in accord with the acidity tests.

In observing moribund forms disintegrate (text fig. C, 1–12), all food vacuoles were seen to be extruded, the body flattened, and pathological vacuoles, largely water, became apparent in the sulcal axis. These ruptured leaving the body very irregular in shape. Successive formation of these vacuoles finally caused complete disintegration of

the body. Even then the nucleus persisted as a unit for some two minutes, when the nuclear membrane ruptured and the karyosome alone remained as a unit, retaining its form for over half an hour. The blepharoplast persisted as an irregular granular mass surrounding the two basal granules. As long as this mass remained as a unit, the flagella could be seen to wave back and forth, but ceased moving as soon as the mass disintegrated.

It is especially noticeable that the body may be distended, elongated, or distorted by newly engulfed food (pl. 9, figs. 19–27). It may elongate to twice its normal length by inclusions of filamentous algae, desmids, or diatoms. Such elongation is usually, though not always, anteroposteriorly. The modifications due to inclusions of such organisms as *Scenedesmus* or *Lagerheimia* I have already mentioned. *Chlorella* and *Protococcus* when engulfed were arranged peripherally within the vacuolated cytoplasm, just underneath the surface reticulum. At times this made the animal appear perfectly green. These frequently popped out through the pellicle and when first observed made me think of zoöids from flagellated forms in multiple fission. Such a condition was but temporary, however, the algae either being digested for food, early showing the surrounding vacuole, or else, I am led to believe, at times assuming the state of transient symbiosis. For three months such a congested condition was both typical and dominant. Seldom was *Chlorella* digested and I am inclined strongly to the idea of transient, or facultative symbiosis.

As seen in the living state, the cytoplasm consists of large, hyaline vacuoles, in the interstices of which are smaller vacuoles, and the spaces between these filled with granules or plasmosomes in a fluid matrix (pl. 8, fig. 5). The periphery of each vacuole seems to consist of a definite membrane, more the result of a turgid surface tension, while the interior is filled with a hyaline fluid.

The surface of the cytoplasm consists of smaller vacuoles with a greater number of granules. The arrangement of these gives the appearance of a surface reticulum, with the larger, deeper vacuoles lying against or within it. When disintegration takes place, the pellicle ruptures, the cytoplasm goes to pieces rapidly, the hyaline fluid diffusing into the water, and the granules, which do not appear nearly so numerous as the mass of the organism might indicate, are scattered by diffusion currents.

The nucleus possesses no vacuoles, but seems to consist, much as the blepharoplast and the immediately surrounding cytoplasm, of granules

in a fixed matrix, denser and more refractive than the cytoplasmic hyaloplasm. In the resting state these are arranged peripherally just within the membrane. The nucleus is usually disc-shaped, being flattened anteroposteriorly. It is, when seen from the front or rear, round, oval, or irregularly elongated. In the living state, and when intravitam stains, such as neutral red and Bismarck brown, are used, there is at the center a karyosome which seems at times to consist of closely compacted granules surrounded by a light hyaline area of nuclear sap in which there are no granules, the periphery of which seems usually to be bounded by a membrane. When the nucleus disintegrates this granular karyosome persists for from fifteen to thirty minutes. When stained with iron-alum haematoxylin, Delafield's haematoxylin, phosphotungstic haematoxylin and others, this karyosome appears homogeneous and this whole mass to be surrounded by the hyaline area, thus giving a perfect vesicular nucleus.

As to the reticular nature of its protoplasm, I am inclined to regard the surface of the vacuoles as modified, perhaps by stress or strain, into thickenings or longitudinal strings of plasmosomes, the interstices of the larger vacuoles being filled with still larger granules of various kinds, food, mitochondria, plastids, metaplastic granules, and foreign organic and inorganic bodies. There is little or no circulation of vacuoles or protoplasm visible in *Collodictyon*. That the nuclear protoplasm has the power to create and absorb or eliminate a membrane, metabolic in character, around the microkaryosome, will be described as a prophase phemonenon; even so, a cytoplasmic vacuole may be bounded by a definite membrane, kinetic in character, which may be modified so as to appear reticular when stained, and on which are accumulated small granules, either scattered or in long, bead-like strings. All of this is in addition to the other granules which may be held in the interstices of both large and small vacuoles.

The physical nature of the protoplasm of *Collodictyon,* therefore, appears to be a fluid, which may be modified by metabolic, kinetic and other life processes into granular or reticular variations, these however, being subject to reabsorption into a hyaline fluid, or becoming by-products, such as plastids, never again functioning in metabolism or life processes, though still retained in the cytoplasm.

Besides the protoplasmic vacuoles, other kinds may be present. 1. Food vacuoles, which may contain plants or animals just engulfed, or which may be very old and alkaline in reaction, simply water or digestive spaces in which little remains. These may flow together

when *Collodictyon* is in a moribund state, giving rise to what Francé calls water vacuoles. 2. Water vacuoles: I can not differentiate these from the old food vacuoles except that they are larger. They give similar reaction with Congo red. Undoubtedly they are pathological, in the sense that they do not appear except in moribund condition. The smaller food vacuoles either are entirely absorbed or their contents are extruded. 3. In the moribund state the simple protoplasmic vacuoles may rupture and flow together, thus creating large degeneration vacuoles, indicative of a quick collapse.

"Contractile vesicles" were seen and described by Carter (1865). He did not figure them, however, and merely indicated that their position was so variable that he evidently failed to locate them for his sketches. Stein (1878–83) described one pulsating vacuole in the anterior end and figured the same. Klebs (1893) indicated one in the posterior end of his organism, which neither Francé nor I believe to be a true *Collodictyon*. Francé (1899) found one at the anterior end, about 6μ in diameter, which pulsated two to three times a minute. He says that it is near the nucleus, but very hard to see on account of the numerous granulations. In all my observations, even with the compound binocular microscope, I have failed to find this vacuole or any other pulsating vacuole. The individuals were often free from inclusions, were studied when actively moving about, when stained intra-vitam with neutral red, Bismarck brown, and methylen blue, when retarded by nicotine, Congo red, anilin solution, litmus solution, weak hydrochloric acid and carbon dioxide. Several times in watching these forms until cytolysis occurred, I have seen the protoplasmic vacuoles flow together, resembling somewhat contractile vacuoles discharging. I am sure these were not pulsating vacuoles. However much I regret to differ from previous observers, especially Francé, who measured and observed the period of pulsation, I am constrained to believe that in the *Collodictyon* of the culture under discussion there is no contractile vacuole.

Francé (1899) sums up most satisfactorily his reasons for believing that there is no cuticle; I agree with him. At the same time, the characteristic form is such, and so constant for the individual, especially for the anterior end, that I am convinced there is a periplast or pellicle, thin and undifferentiated, of smaller vacuoles or homogeneous protoplasm. This must be a coagulation product. It at least is rather impermeable to quick action of many chemicals, especially anilin dyes.

There is no central digestive region, but food vacuoles are held

suspended within the body, at times rupturing or displacing many protoplasmic vacuoles. Small food granules, as *Chlorella,* are arranged peripherally just underneath the periplast, showing that the suspension capacity of the smaller peripheral vacuoles is greater than that of the larger and more centrally located ones. The only evidence of circulation of food vacuoles is that most of the undigested products are evacuated from the posterior portion of the body.

The sulcus has been described in discussing the various modifications of form but its chief features may be here summarized. It cleaves one side, may extend anteriorly so as to cause a cordate or irregular depression in the usual oval contour; posteriorly it may fade out, leaving the general shape conical, or it may divide the body into two cups, thus giving the bifurcated appearance; by secondary branches, which also tend to run longitudinally, there may be produced as many as five posterior cusps. The whole of the sulcal region is amoeboid and functions in food engulfing. Much of the irregularity of shape is due to variations in this region. At its anterior end there is a depression (pl. 9, fig. 19) which may function as a cytostome or esophagus. This seems to end blindly, having no connection with any vacuole. If it may at all be regarded as a cytostome, it is most primitive, more potential and functional than structural.

Collodictyon possesses a true vesicular nucleus, located anteriorly near the base of the flagella and may be either centrally located or displaced, usually away from the sulcus. It is surrounded by a distinct nuclear membrane, from which granular cytoplasm extends out into the body between the protoplasmic vacuoles. The large karyosome is located centrally with a surrounding hyaline area.

The blepharoplast is located anterior to the nucleus, at the base of the flagella and immediately beside the depression caused by the anterior extension of the sulcus. When killed and fixed in strong Flemming and stained in Bordeaux red and iron haematoxylin, the blepharoplast seems to consist of two basal granules surrounded by a more darkly staining granular archoplasm (pl. 9, figs. 23–27). It usually appears, especially when not sufficiently destained, as an irregular chromatic mass in which are embedded the two basal granules which protrude as tubercles, to each of which paired flagella are attached, each also surrounded by a granular archoplasm (pl. 12, figs. 19, 20). It is probable that the irregular chromatic mass is, in fact, simply a lateral view of an archoplasmic plate or cap bounding the granular area and in the center of which are the two basal

granules. There is evidence in vegetative stages for a faint rhizoplast, probably two strands, connecting the blepharoplast to the nucleus, and at times such strands can easily be observed (pl. 8, fig. 13). In division, thickened striations and fibers connecting the blepharoplast and nucleus are more evident than at other times (pl. 11, figs. 40, 44). These no doubt are rightly interpreted as dividing rhizoplasts.

HABITS AND ACTIVITIES

Normally *Collodictyon* is pelagic, floating near the surface of the water in the aquarium, but at all times of the day and at all temperatures tested and under all conditions of light and darkness some have been found scattered throughout the aquarium. In a free drop of water on a slide they tend to stratify near the substrate, but in a hanging drop they swim about throughout the drop, only occasionally accumulating near the slide. In the aquarium they rest both in the direct sunlight and also in shaded portions; but there is a marked tendency to gather nearest the source of light. When there are abundant algae floating on the surface of the aquarium, they can be found at or just beneath the surface and then there is a region of scant distribution below which they tend to accumulate in greatest abundance. This may be on account of a superabundance of oxygen or to too great heat at the surface, due to the absorption of heat by the algae and the surface reflection.

As to the association of *Collodictyon* with water pollution and pools in which decay has been or is progressing rapidly, I am less positive in my convictions than Francé (1899) seemed in his conclusions. My own observations have been that *Collodictyon* can not live where there is a great amount of decay. They are holozoic, however (with the possible exception of times when there is a symbiotic association with *Chlorella*), and live on Protozoa and algae which are associated with decay. Their own life seems far removed from saprozoic nutrition and I find little in the rate of multiplication that tends to confirm such a supposition or conclusion. As to the fact that they were found in pools where disintegration was rapidly increasing or at a maximum, I am not in a position to question except from cultural experiments, in which other factors might have played a determining part; but I urge this same factor, unknown as it is, in explanation of Francé's observation. Francé's argument that *Euglena* is its chief and only

source of food will not hold, since I find engulfed *Ulothrix,* dinoflagellates, *Pandorina, Gonium, Chlorella,* etc., more common in my aquarium than *Euglena.* I do find the species collectively a "lover of pure water," thriving in sunshine and in a balanced aquarium. I can therefore, at least conclude that increasing or maximum decay is not essential to the life of the organism, and that *Collodictyon* is not a determining factor in water pollution.

When free swimming, *Collodictyon* moves forward by beautiful lashings of the flagella in true tractellar style, the flagella undulating as the animal circles about. It may also move backward by the anterior adaxial action of the flagella, but only seems to do so in an avoiding reaction. It rotates on its longitudinal axis more frequently clockwise, but seemingly without cause or provocation may reverse and rotate counter-clockwise. The flagella may beat back on all sides of the body, closely appressed to the pellicle. It frequently, when near the substratum, attaches itself by its flagella and rotates clockwise about its longitudinal axis. As to the explanation of this I am in doubt. I am inclined to believe it simply a thigmotactic response, possibly combined with positive geotropism; but in swimming through the water when nearing an object it touches it with its flagella and usually passes to one side or jostles the object out of the way if small enough.

In its feeding habits, *Collodictyon* is most interesting. When hungry, it can be distinguished from moribund stages in which all food is extruded by pseudopodial projections from the lateral groove or sulcal region (text fig. A, 1–5). Francé emphasized the adhering engulfing process, speaking little of the pseudopodia. I wish to emphasize these pseudopodia, for I observe that they function actively whenever the organism is seeking food. At these times when coming in contact with Protozoa or algae which it may use for food, they are wafted to the sulcal region by the flagella, or else *Collodictyon* aligns itself alongside of its prey with the pseudopodia in contact. If an elongated filamentous alga is to be engulfed, the relationship between the two is nearly always with the alga lying in the groove longitudinally; but I have noticed with diatoms that they just as frequently are engulfed by the end. Both the flagella and the pseudopodia appear sensitive to food stimulus and usually there is coördination between the protoplasm of the sulcal region and the flagella, though there seems to be no mechanism for this other than the primitive characteristics of the protoplasm. The process of the organization of a food vacuole is a combination of circumvallation and

circumfluence (Minchin, 1912, p. 189). The food is frequently surrounded by engulfing protoplasm of the pseudopodium before it begins to sink into the vacuolated body, but the latter process always takes place and at such times there may be a considerable shifting of internal vacuoles. Once I noticed the rupture of several vacuoles on the engulfing of a very large *Pandorina morum,* undoubtedly explicable by the movements of the captured organism. *Collodictyon* may engulf much food, almost as much as its own size and still appear very little larger. Francé cites an instance where ten *Euglena minima* and *Chlamydomonas* filled up the interior of *Collodictyon.* Its normal cytoplasmic vacuoles must, therefore, not only be displaced, but also ruptured and the food vacuole take the place of one or more of these. There is always a slight water ring surrounding the new food vacuole, but as it grows older this seems to be supplanted by a protoplasmic film coming directly in contact with the substance being digested. Tests with Congo red and litmus bring out these differences intra-vitam. In the use of the former, the food vacuoles present, for some time appear red, thus indicating alkalinity, but the small vacuoles in final stage of digestion are blue, indicating acidity. Litmus did not yield such good results, though the water film was shown up very well. *Collodictyon* has been seen to be engulfed by a larger form of its own species. It is not only a cannibal, but is very voracious, and almost omnivorous. *Peridinium, Pandorina, Euglena, Amoeba, Chlamydomonas,* a ciliate (presumably *Colpidium*), *Pediastrum, Scenedesmus, Lagerheimia, Ulothrix, Chlorella, Navicula,* and *Gonium,* have been observed being ingested or in food vacuoles within the body (pl. 9, figs. 19–27).

MITOSIS

Resting Stage

In the normal nucleus of *Collodictyon* in the resting stage the following organelles appear to play important rôles. The *nucleus* is surrounded by a *nuclear membrane,* which stains very lightly with iron haematoxylin, but a dark red with acid fuchsin and safranin. The shape of the nucleus is variable but typically is an ovoid flattened on the posterior side or anteroposteriorly, the longitudinal axis lying perpendicular to the major axis of the cell. This nucleus is *vesicular.* The central *karyosome* measures 2 to 3μ in diameter and appears homogeneous with all stains except Bordeaux red, iron haematoxylin, and neutral red used intra-vitam, with which it appears granular;

with safranin, the periphery appears to contain masses of granules of chromatin while the center is homogeneous and more or less translucent. Surrounding this karyosome is a *hyaline area,* measuring from 1 to 3μ in width, which is always transparent and takes none of the nucleus stains but is lightly colored with eosin and acid fuchsin about the same as the cytoplasm. Around this there is a *peripheral area,* varying in width and definiteness, in which irregular *chromatin masses,* small, variable in size, number and shape, occur. This area is from $\frac{1}{2}$ to 3μ in width. It is surrounded by the nuclear membrane.

The *chromatin* material is frequently encrusted upon the nuclear membrane in the resting stages of the nucleus. Much chromatin is accumulated in masses scattered peripherally between the hyaline area and the membrane, at times (presumably when anabolic processes greatly predominate over the katabolic) reducing the hyaline area to a minimum. But the largest amount of the chromatin is found in the karyosome which stains deeply with all nuclear stains. Thus the chromatin encrusted upon the membrane, that occurring in the peripheral zone, and that making up the karyosome, or the larger part of it, all has to be accounted for later in mitosis.

The *blepharoplast,* located at the anterior end, is irregular in size and shape. It consists of a mass of chromatoidal protoplasm which tends to become stellate in shape, much like a nerve cell, deeply staining strands extending out into the cytoplasm between the vacuoles (pl. 9, fig. 20). Embedded in this blepharoplast are two *basal granules,* which are distinctly red when stained with safranin, gentian-violet, orange G, and acid fuchsin. From each of these basal granules two equal flagella arise. From the blepharoplast, probably from each of the basal granules, arise the two *rhizoplasts,* which extend as strands from the chromatoidal mass, but instead of remaining upon the surface as the other strands do, run interiorly to the nucleus, enlarging at the nuclear membrane into a minute granule. In some instances the nuclear membrane is drawn up at the point of attachment of the extranuclear rhizoplast. In several instances (pl. 7, fig. 1), an intranuclear rhizoplast seems to penetrate the membrane and run to the central karyosome.

The cytoplasm immediately surrounding the nucleus is closely appressed to the membrane, making it difficult at times to distinguish the latter. It is denser and more granular, and extends out into the body in strands which lie between the protoplasmic vacuoles. It stains much as the peripheral nuclear area with iron haematoxylin,

but is not so evident with safranin, gentian-violet, orange G, or Mallory's modified connective tissue stain. This area of cytoplasm is not differential cytoplasm, for the interstitial material between or bounding the protoplasmic vacuoles throughout the cytoplasm stain thus deeply with all nuclear as well as plasma stains that I have tried. It seems, therefore, to be merely the cytoplasm in which the nucleus is suspended, being largely granular instead of vacuolar. This denser cytoplasmic area just surrounding the nucleus is a condition of the resting nucleus and not a mitotic phenomenon.

The state of the microkaryosome is of help in determining the progress of mitosis especially of the prophase; but the expanding of the kinetic membrane is the best criterion of this. A chromatin halo inside of the nucleus usually accompanies this.

When division begins, or very soon thereafter, all undigested food particles and other foreign bodies are extruded. There is no rounding up of the cell, the characteristic shape being retained throughout mitosis. I have never seen pseudopodia or amoeboid protrusions from the sulcal region in division stages.

Unequal Constriction of the Karyosome

Preliminary to true mitosis, the karyosome usually elongates and constricts into a dumb-bell shape with the knobs of unequal size. These pull apart until connected only by a strand, which finally breaks, accomplishing an unequal or differential division of the karyosome (pl. 10, figs. 29, 30). The resulting large and small daughter karyosomes are not equivalents either in size or behavior, and by reason of their size I shall designate them macrokaryosomes and microkaryosomes respectively (pl. 10, figs. 31, 32).

During this preliminary unequal constriction of the karyosome, I have been unable to detect any change in the blepharoplast, which consists of two basal granules surrounded by granular archoplasm. I have not seen anything resembling a splitting of the rhizoplast at either end. The flagella are still four in number at this stage (pl. 10, figs. 28–31, 34, 36).

The macrokaryosome is homogeneous in appearance with all stains used and contains no residual body. It may be regarded as consisting of plastin impregnated with chromatin. Its behavior is passive. It loses its surrounding hyaline area, and may round up or form a crescentic mass around and outside of the kinetic membrane. It is

then pushed by this expanding membrane out to the periphery of the nucleus, where it reposes in a niche of the nuclear membrane, but separated from the mitotic area within by the persistent kinetic membrane (pl. 11, figs. 38, 44).

It has been found on several occasions (pl. 11, fig. 43) to break up and form an intranuclear chromatin cloud. Even where it has been traced repeatedly to its position near the nuclear membrane, I have never located it or any similar chromatin mass outside the nucleus. In some instances it seems that the cytoplasm just around the nucleus is more deeply stained by a chromidial cloud as though the macrokaryosome or a part of it were extruded in fine granules. Such is undoubtedly its fate in many individuals. But much evidence points to the gradual absorption of the macrokaryosome *in situ* in some instances within the nuclear membrane, with the formation of an intranuclear, intrakinetic membrane chromatin cloud (pl. 10, figs. 35, 36, pl. 11, figs. 37, 38, 41, 42, 44, 45). There is some evidence (pl. 11, fig. 46, pl. 12, fig. 48) that it may in other instances persist and pass over to one of the daughter nuclei without complete dissolution. There is no evidence for its splitting or division on the equatorial plate. Its behavior seems dependent in some way upon the metabolic equilibrium of the nucleus and cytoplasm. Its significance, regarding possible relations with the parabasal body of parasitic flagellates, the macronucleus of ciliates, Hertwig's theory of trophochromatin and idiochromatin, and Hartmann's binuclear theory, in so far as flagellates are concerned, will be taken up in subsequent discussion.

Individuals in evident stages of the prophase have been observed in which there is a total absence of any evidence of a constriction of the karyosome. The kinetic karyosome (microkaryosome) entering into mitosis may likewise on rare occasions be larger than the other mass, the passive macrokaryosome.

Mitosis

With the organization of the microkaryosome mitosis begins. Binary fission by longitudinal division seems to be initiated within this karyosome. This forms about itself the kinetic membrane, which continues to expand until it becomes commensurate with the nuclear membrane. The faint rhizoplasts extend from the kinetic membrane which surrounds the organizing microkaryosome, through the

peripheral granular area to the nuclear membrane; from here they pass through the intervening cytoplasm to the basal granules within the blepharoplast. The rhizoplasts are not sufficiently prominent to be evident to the uninitated eye. They may easily be confused with the attenuated strands from the blepharoplasts radiating out into the surface cytoplasm.

Two small granules have been observed just at the point where the rhizoplasts enter the nuclear membrane on their way to the central karyosome (pl. 8, figs. 9, 13). In early and late prophase stages (pl. 14, fig. 75) these separate and the nuclear membrane appears heavier or thicker between these points in comparison with the rest of the membrane, as though an extranuclear paradesmose were forming. In a lately discovered metaphase (pl. 14, fig. 78) this paradesmose is well formed, connecting the polar ends of the spindle. In one particularly favorable anaphase (pl. 14, fig. 83) granules of considerable size are located at the polar ends of the daughter chromatin masses and these are connected by a heavy paradesmose upon the nuclear wall.

This evidence points definitely to the presence of an extranuclear division center or centrosome. Such is typical of parasitic polymastigotes (Kofoid and Christiansen, 1915, Kofoid and Swezy, 1915*a*, 1915*b*, Swezy, 1915, Boeck, 1917) and may be considered typical of polymastigotes in general. In this case, however, the blepharoplast and centrosome are separate, the latter adhering to the nuclear membrane.

The rhizoplasts split first at the end near the nuclear membrane, presumably with the division and separation of the centrosomes. The split extends anteriorly, giving the appearance of a V-shaped striated region (pl. 8, fig. 13). Finally with the separation and division of the basal granules of the blepharoplast (pl. 8, fig. 9) the rhizoplasts appear distinct and their points of contact with the nuclear membrane become farther apart.

In justice to truth, it must be said that the problem of the division center has been full of difficulties. The material in hand is of such a nature that the possibility of error must not be overlooked. The apparent points of contact of the rhizoplast with the nuclear membrane are exceedingly faint. The extranuclear cytoplasmic granules and vacuoles and the intranuclear chromatin encrusted in granules upon the nuclear membrane, which are connected in prophase by somewhat chromatic lines, are exceedingly confusing. This renders

the above interpretation still tentative. Much work is still needed upon *Collodictyon* and related forms to clear up fully the question of the division center.

The time at which the blepharoplast divides has not been definitely determined. There is evidence of its division as early as the middle of the prophase (pl. 11, figs. 40, 40*a*). It seems, from the figures just referred to, that the method is one of doubling of the basal granules, thus making two pairs, which gradually separate (pl. 11, fig. 46, pl. 12, figs. 48, 53, 54). No splitting of the flagella has been observed, but since only equal flagella have been found in these stages, it seems safe to conclude that the flagella split longitudinally or new ones grow out at about the same time as the doubling of the paired basal granules. At the metaphase the doubling is complete (pl. 12, fig. 48).

PROPHASE

With the unequal constriction of the karyosome, sometimes even before this differential division has been completed, the microkaryosome organizes about itself a membrane, which seems to have a kinetic or metabolic function and which I shall designate as the *kinetic membrane*. The macrokaryosome is passive in behavior and remains outside of this active membrane. The kinetic membrane does not simply bound the hyaline area, but is almost surrounded by such a zone (pl. 10, fig. 32). At first the space between the microkaryosome and the membrane is hyaline, but it is soon filled with a dense chromatin cloud (pl. 11, figs. 37, 38, 41, 42, 44). This kinetic membrane seems somewhat less chromatic than the nuclear membrane. Usually it is spherical or irregularly globular, but in two or three instances angular, almost polygonal (pl. 10, figs. 32, 33), which is probably an artifact. As it enlarges, however, it may elongate and its shape become modified by the organizing spindle.

When the organization of the microkaryosome is begun, the chromatin outside the kinetic membrane tends to accumulate in granules or masses which are linked together by slightly chromatic strands (pl. 10, figs. 30, 32, 36). Much of it is encrusted upon the nuclear membrane (pl. 10, fig. 32); much forms immediately around the hyaline area surrounding the kinetic membrane (pl. 10, fig. 30), frequently also about the macrokaryosome (pl. 11, fig. 40). The whole peripherial zone becomes thus involved and with the expansion of the active hyaline area is more and more encroached upon until

only the chromatin encrusted upon the nuclear membrane remains outside of it. As to the behavior of this peripheral chromatin, there is some doubt. It may pass into solution and become a part of the metaphase chromosomes, or spread out upon the nuclear membrane, making this appear heavier and darker, or it may be extruded into the cytoplasm. There is an indication in a few individuals of a peculiar splitting of peripheral encrusted chromatin bodies (pl. 11, fig. 46). I can not verify this as a regular occurrence nor can I regard it as typical. This splitting is prior to the metaphase and may be closely correlated with the precocious splitting of the segmented spireme (pl. 12, fig. 47).

The microkaryosome elongates and divides, the separation being characterized by connecting fibrils somewhat resembling spindle fibers, rather than a dumb-bell constriction as in differential division of the primary karyosome (pl. 10, figs. 34, 35, 36). A chromatin cloud forms immediately around this elongating microkaryosome and fills up the intervening space, almost obliterating the fibers (pl. 11, figs. 37, 38). This cloud expands until the whole area within the kinetic membrane becomes diffusely filled with fine granules.

This early microkaryosome organization is exceedingly complex for microscopic analysis, but it soon becomes evident that instead of having divided simply into two masses, the microkaryosome is undergoing a segmenting process (pl. 11, figs. 39, 40, 41, 42, 44, 45), preliminary to a final prophase spireme (pl. 12, fig. 47). The first division is followed by a second elongation of each mass, apparently passing through a tripod and ring stage, in time forming two crescentic masses or a segmented skein (pl. 11, figs. 40, 41, 42, 44). This retains terminal chromatin masses or knobs which probably form the basic elements of the future chromosomes. The next phase is a longitudinal splitting of each segment (pl. 11, fig. 45). If all terminal knobs divide at this time, eight chromatin masses would result and this would probably determine the correct count of chromosomes. It is possible that one of the four terminal knobs fails to divide, and this would give but seven chromatin masses—as many chromosomes as I have been able to count (pl. 12, fig. 50). Such a phenomenon is common in mitoses of higher animals (Wenrich, 1916; Carothers, 1917), but it must be admitted that the evidence here is not conclusive (pl. 11, fig. 45). The middle and final prophase stages are characterized by an active organization of chromatin upon the segmenting skein. That there is some chromatin in the original

microkaryosome there can be little doubt and this seems to persist as the terminal knobs to the skein segments.

That all the chromatin entering into the formation of the chromosomes can not possibly be of the original microkaryosome is obvious. Considering the average individuals, the average size of the microkaryosomes when separated from the macrokaryosomes may be estimated at about 1.5 to 2μ in diameter. The size of six of the chromosomes may relatively be estimated at $0.75 \times 1.5\mu$ each, the small one being only half as large (pl. 12, fig. 50). All other chromatin is outside the kinetic membrane and it does not seem probable that it could go through as granules or mass units, since the area immediately around the kinetic membrane is hyaline and in a state of solution, while the area within becomes filled with a dense chromatin cloud, and the only chromatin masses consist of the persisting and reorganizing elements of the microkaryosome. It appears, therefore, that there is a solvent action within the hyaline area around the membrane and that chromatin from the granules in the peripheral zone, possibly from the macrokaryosome, and probably from that encrusted upon the nuclear membrane, is dissolved and passed through the kinetic membrane by diffusion and enters into the composition of the chromosomes. This demands a higher pressure from without, which can easily be accounted for by the chromatin within the membrane being condensed and precipitated upon the achromatic linin fibers of the skein. When the kinetic membrane has expanded to the limits of the nuclear membrane, this early phase of a segmented spireme (pl. 12, fig. 49) finally organizes into a skein resembling a more or less continuous ribbon, with chromatin granules embedded upon it. This seems to be accomplished by a longitudinal separation of the segments. the terminal knobs especially showing this division. A final split involving the formation of sixteen chromatin masses (pl. 12, fig. 49) may be found and may be regarded as the precocious splitting of the definitive chromosomes. From these chromatin masses which are already arranged in an equatorial belt the seven or eight chromosomes of the equatorial plate are finally organized (pl. 12, fig. 50), probably re-fused by a telosynaptic process.

The interpretation of a segmenting spireme seems necessary because of the fact that its elements seem to be directly produced by the early division and organization of the microkaryosome, this being evident before the metabolic membrane has expanded to its final proportions. This spireme frequently has the appearance of a

tripod, ring, or double crescent (pl. 11, figs. 39, 40, 41, 42, 44). I found one difficulty of technique here hard to overcome. The chromatin cloud is usually so dense that whenever this is sufficiently destained to see the skein, this latter is rendered unfit for detailed interpretation.

In *Phrynotettix* (Wenrich, 1916, p. 112) plasmosomes may change into polar granules of chromosomes and vice versa.

> One of the most puzzling problems that cytologists have to deal with is the behavior and function of the so-called "plasmosomes" or "nucleoli." They apparently exhibit such a varietv of reactions to methods of technique, and exhibit such varying relationships to other structures in the cell, that it is almost hopeless even to attempt to classify them. That they play some important rôle in the physiology of the cell, there is not the slightest doubt, but what that rôle is, or what relation they bear to the question of chromosome-individuality, are problems that are far from a solution at the present time.

There is hardly a close analogy between *Phrynotettix* and *Collodictyon*, but here they offer a most interesting comparison. The terminal knobs of the segmenting skein of *Collodictyon* lend themselves to the interpretation that they are elements at least of the chromosomes. In *Phrynotettix* the individuality of the chromosomes is traced by similar chromatin masses which, however, are not all terminal and several of which may enter into the composition of a chromosome.

The blepharoplast divides during the middle or final prophase. The rhizoplasts thicken and evidently split soon after the kinetic membrane begins to expand (pl. 11, fig. 40). The spindle is not organized until after the spireme and skein are far advanced. The cytoplasm immediately surrounding the nucleus may become darker, due to the presence of a chromidial cloud from extruded chromatin. The nuclear membrane persists. The protoplasmic vacuoles may or may not be large. They are as normal as in the vegetative stages and their variations are largely the result of food vacuoles extruded at the beginning of division. If one so cares, the prophase may be regarded as beginning with the unequal constriction of the karyosome. I have separated these stages arbitrarily for advantages of analysis.

METAPHASE

The nucleus becomes filled with a perfect spindle, which lies at right angles to both the major axis of the cell and to the sulcal axis, usually on one side or the other of the sulcus. The chromosomes are now seven or eight in number (pl. 12, fig. 50). There is a general

uniformity of size and shape, being ovoid, from 1.5 to 2μ in length, 0.5 to 0.75μ wide; with one exception, namely that one chromosome is but half so long and hardly so wide. These are arranged equatorially with very slight tendency to V-shape or crescent-shape. Their elongated axes are meridional to the spindle.

In division (pl. 12, fig. 50) all the chromosomes but one in this spindle appear to have parted transversely. The spindle fibers are attached to the ends and not to the centers or sides. This parting may be fundamentally similar to the parting of the chromosomes as found by Tschenzoff (1916) in *Euglena*. The precocious splitting is evidently not so far removed from the metaphase, however, since in *Euglena* it apparently takes place in the preceding telophase of the parental individual, while in *Collodictyon* it takes place no farther back than the late prophase just preceding the metaphase.

In the most satisfactory metaphase I have found (pl. 12, fig. 50), the small chromosome has not as yet divided. No constriction can be found in it in this figure. It may best be interpreted as a lagging chromosome. In such a case it would probably divide later on in the metaphase or early anaphase, though there is no available material to determine this matter.

In one anaphase (pl. 12, fig. 51) there are obviously unequal chromatin masses. These are either so deeply stained as to prevent an accurate chromosome count or else the chromosomes were contracted and massed in the killing and fixation. It is barely possible that this inequality of mass is due to the failure of the lagging chromosome to divide, thus giving an unequal qualitative as well as quantitative division comparable to the sex chromosome; but we have as yet no evidence of gamete formation in this genus. Such inequality may also be explained by the passing over to one of the daughter nuclei of the remnants at least of the macrokaryosome (pl. 12, figs. 48, 56). Any inequality of mass which is evident in early anaphase is soon obscured by the growth of chromatin, which is proceeding rapidly. I regret that I have not found a sufficient number of metaphase stages to warrant a detailed study. Of the many thousand individuals studied I have seen but three or four equatorial plates.

As suggested above, the indication of a peculiar splitting of certain peripheral chromatin granules is a prophase, not a metaphase phenomenon, related rather to the precocious splitting of the segmented spireme. I have found no indication of a division of the

macrokaryosome at the time of the splitting of the peripheral granules, or when it persists and is found on or near the equatorial plate (pl. 11, fig. 46). As noted above, it may (pl. 12, fig. 48) pass undivided to one of the poles and thus to one of the daughter nuclei (pl. 12, fig. 56). This would explain the inequality of the anaphase chromatin masses. It may disintegrate (pl. 11, fig. 43), go into solution, and finally enter into the composition of the chromosomes. It may, which is very probable, pass from its niche in the nuclear membrane, out of the nucleus to form an extranuclear chromidial cloud or simply be dissipated or absorbed into the cytoplasm. It was found to divide into two unequal segments in several instances in the early prophase (pl. 10, fig. 33), but it is hardly probable that much significance attaches to this, since it is not coördinated with chromatin divisions elsewhere.

ANAPHASE AND TELOPHASE

After the transverse splitting and separation of the chromosomes, each daughter group passes toward its respective pole. When only slightly separated, the chromosomes fuse into a densely staining mass (pl. 12, figs. 52, 54, 55), but can hardly be said to lose their identity here, since knobs and masses resembling ends of chromosomes protrude irregularly from the mass. These chromatin masses become organized into a skein or spireme, a great number of small granules arranged on linear linin threads, before the nuclear membrane has divided (pl. 12, fig. 53). The spindle fibers still persist (pl. 12, fig. 52) after the daughter chromatin masses have drawn near their respective poles, but are only slightly visible at the former equatorial plate. The nuclear wall constricts and nuclear division is accomplished, with the chromatin still in compact masses.

As the daughter chromatin masses pass to their respective poles, they increase perceptibly in size until each equals or exceeds the size of the original karyosome (pl. 12, figs. 52, 55). This is evidently growth and not concretion or deposition, since the chromidial cloud practically disappears at the metaphase and then deepens again in the anaphase.

Toward the final anaphase the chromatin mass or skein breaks up into numerous chromatin masses scattered irregularly through the daughter nuclei. A cloud seems to fill the nucleus and spreads to the surrounding cytoplasm, indicating excessive metabolic activity.

The plane of division runs parallel to the major axis and the sulcus or chief longitudinal groove. The daughter blepharoplasts separate and move to either side of this plane. The basal granules divide earlier in the prophase, as do probably also each of the two flagella, thus producing for each daughter blepharoplast two basal granules and four flagella. I find no stages in which there are shorter or unequal flagella, indicating outgrowth of new flagella, and yet no evidence of splitting has been observed.

The major sulcus deepens and in this plane there is a complete peripheral cytoplasmic constriction (pl. 13, figs. 57–60). The cytoplasm rounds itself up and the daughter organisms are then held together by a thin, highly vacuolated, protoplasmic connection, containing one or more large vacuoles. Division is finally accomplished by the rupturing of these vacuoles. At the time of final separation, the chromatin masses are scattered irregularly through a clouded nucleus. Part of these mass together, round up into a karyosome on which is deposited the immediately surrounding cloud, thus leaving a hyaline area. A large part of the chromatin remains on the outside of this hyaline area and tends to dissassociate, forming the peripheral granular area (pl. 13, fig. 59). There is great metabolic activity at this stage, for the whole cell, especially the anterior end, is darkened by a chromidial cloud. At completion of nuclear reorganization the rhizoplasts assume their small, almost invisible appearance and the typical vegetative organism results.

SUMMARY OF OBSERVATIONS

1. Verification of the work of Carter (1865), Stein (1878), and Francé (1899).

2. Failure to find a contracting vacuole, a point upon which previous observers are at variance.

3. Determination of a fundamental polarity and a superficial symmetry, with anterior and posterior, sulcal and absulcal areas.

4. There is evidence of a very primitive cytostome just at the base of the flagella. The sulcus itself may be regarded as an extension of the cytostome.

5. The blepharoplast consists of two basal granules surrounded by a granular archoplasm. When not sufficiently destained, it resembles a more or less branched and attenuated mass, from which

two tubercles protrude. From each granule two equal flagella arise. Two faint rhizoplasts join the blepharoplast to the nucleus and karyosome. At the points of contact of the rhizoplasts with the nuclear membrane, very small granules are found, which function as extranuclear centrosomes.

6. The typical vesicular nucleus undergoes a true mitosis of a type probably related to mesomitosis.

7. There is an unequal constriction and differential division of the initial karyosome, the resulting karyosomes being designated macrokaryosomes and microkaryosomes.

8. A kinetic membrane is organized around the microkaryosome and during the prophase expands until it apparently becomes commensurate with the nuclear membrane.

9. The macrokaryosome rounds up in a niche of the nuclear membrane, not being involved in mitosis. Its possible fate and significance will be discussed in the latter part of this thesis.

10. The nuclear membrane is persistent during mitosis.

11. There is evidence for an extranuclear centrosome, such as has been found in parasitic flagellates, but in this instance it is separated from the blepharoplast and connected with the same by rhizoplasts. Further work on this and other free living flagellates is much needed to more clearly demonstrate this and related problems.

12. There is an intranuclear chromatin cloud during the prophase. In the final prophase, the anaphase and telophase an extranuclear chromidial cloud is also formed.

13. The microkaryosome organizes within the kinetic membrane, apparently separates into two masses connected by fibers and may pass through a tripod and ring stage. This passes into a double segmented spireme stage, in which there are four terminal chromatin masses or knobs.

14. A separation of this spireme takes place, the resulting terminal masses, seven or eight, forming the chromosomes. There is a precocious splitting of the peripheral chromatin granules.

15. In the final prophase when the segmented skein is arranged about the equatorial plate, there is a precocious splitting of the chromatin masses, which may be indicative of the division and distribution of the chromosomes which is about to take place. In this way the transverse division of the chromosomes may be explained as a fundamental longitudinal division, as determined by this precocious splitting.

16. The number of chromosomes is seven or eight, which in metaphase are arranged on the equatorial plate of a perfect spindle.

17. The chromosomes part transversely. In the only satisfactory metaphase observed (pl. 12, fig. 50) one lags on the spindle.

18. The resulting chromatin masses in early anaphase are sometimes unequal in size, but this is soon concealed by a rapid growth of chromatin in the later anaphase and telophase.

19. The reorganization of a typical skein, which breaks up into chromatin masses, some of which go to form the karyosome, some the peripheral chromatin, and some may be extruded.

20. The basal granules separate, the flagella split longitudinally or grow out anew, the rhizoplasts split from the nuclear end, and the two resulting blepharoplasts contain two new basal granules from division of one of the old, inherited from the old blepharoplast, and are connected with four equal flagella and a rhizoplast.

21. A paradesmose typical of polymastigotes in general is present between the separating centrosomes, on the nuclear membrane.

22. Final separation of the cells takes place in the plane of the sulcus, parallel with the major axis of the cell, by the rupture of one or more of the vacuoles of the constricted protoplasmic connection.

DISCUSSION

Classification and Relationship

Prowazek (1903*a*) attempted to classify the nuclei of Flagellata, distinguishing four different types, which Dobell (1908) has summed up:

1. Simple nuclei, with an evenly distributed chromatic network, and no internal structures (karyosome, division centre, etc.), e.g., *Herpetomonas.*

2. Vesicular nuclei, with direct division; with central chromatin mass surrounded by a clear zone, across which a more or less distinct network extends outward to the nuclear membrane. Such a nucleus may be seen in some species of *Bodo,* and is well seen in *Copromonas.*

3. Centronuclei containing a "nucleolo-centrosome" (Keuten, 1895) and separate chromatin masses. This type of nucleus is characteristic of *Euglena* and its allies. (The centro-nucleus, as defined by Boveri, is a nucleus which contains a cyto-centre, either in a consolidated or diffuse form. In the case of *Euglena,* etc., the cyto-centre is the nucleolo-centrosome, i.e., is of the consolidated type.)

4. Vesicular nuclei with karyokinetic division: e.g., *Polytoma, Chlamydomonas,* etc.

To these Dobell added a fifth:

5. Nuclei in which the achromatic division-centre lies freely in the cell, whilst the chromatin is diffuse in the form of chromidia.

In such a classification *Collodictyon* finds no place. Recent work of Tschenzoff (1916) on *Euglena* and of Bělař (1916) on *Astasia* show related nuclear phenomena which furnish an interesting comparison with *Collodictyon.* These possess a "nucleolo-centrosome" (Keuten, 1895), the significance of which seems not to be well understood. In division it presents the appearance of a centrodesmose. *Collodictyon* possesses no such evident body or nucleolo-centrosome, but there is evidence of an extranuclear centrosome similar to that in other polymastigotes. There is a much more perfect spindle and all chromatic material of the nucleus in the metaphase is located upon the equatorial plate. It thus seems to have a more advanced type of mitosis than do the above-mentioned Euglenoidea. With the present advancement in the science of protozoology it would seem possible to review the various groups of flagellates already recorded, not only describing their typical vegetative, but their mitotic phenomena, and to establish complete life cycles for the majority. But such is not the case. All previous investigations of *Collodictyon,* to use it as an example, have omitted description and discussion of mitotic and related phenomena. It is hoped that this knowledge has been brought out in sufficient detail and accuracy to warrant future correlations and comparisons, though little more than a beginning is claimed to have been made.

Because of its plastic nature and its mode of incepting food, "for it does not appear to possess any oral aperture," Carter (1865, p. 289) classified *Collodictyon* as a rhizopod. He, however, acknowledged its similarities to *Bodo* Ehr., in its voracity especially, to *Polyselmis viridis* Duj., and to *Actinophrys eichornii* in its vacuolated cytoplasm, the "cellular spaces which pervade its body." These comparisons have little significance today.

Stein (1878), naming it *Tetramitus sulcatus* Stein, placed it in the flagellate group and in the first family, Monadina, together with the genera: *Cercomonas, Monas, Goniomonas, Bodo, Phyllomitus,* (*Tetramitus*), *Trepomonas, Trichomonas. Hexamitus, Lophomonas,* and *Platytheca.*

Kent (1880–81) interpreting Carter's and Stein's organisms as different, put both under Order IV, Flagellata-Pantostomata; *Collodictyon* in Family XIV, Trimastigidae with *Trichomonas, Dallingeria*

and *Trimastix; Tetramitus* in Family XV, Tetramitidae with *Tetraselmis* and *Chloraster.* He compared the vacuolated cytoplasm to that of *Noctiluca, Leptodiscus, Trachelius* and *Loxodes.*

Bütschli (1883–87), recognizing *Tetramitus sulcatus* as the synonym of *Collodictyon,* characterized it briefly under the Family Tetramitina of the Suborder Monadina Bütschli, with *Tetramitus* Perty, *Monocercomonas* Grassi, *Trichomonas,* and *Trichomastix,* and placed the Polymastigina as the succeeding family with the genera *Hexamitus, Megastoma,* and *Polymastix.*

Francé (1899, p. 19), after discoursing on the inadequacy of the classification of flagellates, says:

> Man eröffnet einfach systematische Rumpelkammern, in die man der "Urväter Hausrath drein gestopft." Dort liegen sie, ein trübseliges Chaos von Bodoninen, Monadinen, Dendromonaden, mit denem man nichts anzufangen weiss. Von dort holte ich mir auch meine *Collodictyon,* deren Bau und Lebensgeschichte darzulegen, die Aufgabe der folgenden Zeilen ist.

In concluding his paper (p. 26) he wrote:

> Ich begrüsste die generische Selbständigkeit mit umsomehr Freude, als ich der Ansicht bin, *Collodictyon* sie mit den viel höher organisierten Tetramitiden gar nich näher verwandt. Es ist eine sehr primitive Zelle, welche nach Art der Monadinen gebaut ist, so lebt und sich sowie sie vermehrt. Höhere Differenzierungen besitzt es gar nicht, sondern nur lauter solche charaktere, welche es erfordern diesen Organismus den Monadinen anzugliedern. Damit wäre aber mein anfangs gestechtes Ziel erreicht, diesem Wesen endlich seinen dauernden Platz im System anweisen zu kennen.

There is little agreement among taxonomists since 1900 as to the group with which *Collodictyon* is associated. G. Senn (1900), probably as consistent as any, placed it in the Order Protomastigineae (coördinate with I. Pantostomatineae, III. Distomatineae, IV. Chrysomonadineae, V. Cryptomonadineae, VI. Chloromonadinae, VII. Euglenidae), in the ninth and last family, Tetramitaceae, with *Costiopsis, Tetramitus,* (*Collodictyon*), *Trichomastix, Trichomonas,* and *Polymastix.* Lemmermann (1910) follows Senn, accepting *Costia* instead of *Costiopsis.*

Klebs (1893) and Blochmann (1895) accept five orders: Protomonadina, Polymastigina, Euglenoidea, Chromomonadina, and Phytomonadina, including *Collodictyon* in the Polymastigina.

Hartmann and Chagas (1910) put the Protomonadina and the Polymastigina into a single order, the Protomonadina, and add the

orders Rhizomastigina and Binucleata (Rhizomastigina, Protomonadina, Binucleata, Chromomonadina, Euglenoidea, Phytomonadina). This is the system adopted by Hartmann.

Calkins (1909) and Lankester (1909), as did Delage and Hérouard (1895), put *Collodictyon* in the tribe or subtribe Monostomatina, organisms with mouth opening at the base of the group of from four to six flagella, as contrasted with the Astomea, which have no special mouth openings. The sulcal region of *Collodictyon* may indeed be regarded as a cytostome. In this structure, however, we have an extension of the cytostome as a metabolic surface. Its pseudopodia mark it as a generalized rather than a specialized type. *Collodictyon* may thus be regarded as comparable with organisms like *Mastigamoeba* Schultze, *Cercomonas crassicauda* Dujardin, and *Tetramitus* or *Trichomonas*. Regarding the above classification, therefore, the validity of the tribes Astomea and Monostomatina is lessened or *Collodictyon* must be regarded as an intermediate type.

Neither Doflein in his *Lehrbuch* (1911) nor Minchin in his *Introtroduction to Protozoology* (1912) classify *Collodictyon*. Senn, Klebs, Doflein, and Minchin, however, all accept the Order Polymastigina, into which *Collodictyon* naturally falls. Klebs and Doflein contrast this with Protomonadina, Minchin with Pantastomina and Protomonadina, Senn with Pantastomatineae and Distomatineae. These distinctions by various authors are not so much opposed to one another as it at first seems. All accept the number of flagella as of determining value. It is very desirable to have no all-inclusive taxonomical groups such as the older Monadina. At the same time artificial distinctions do not tend to clarify the situation. Hartmann was evidently actuated by such a feeling when he combined the Polymastigina with the Protomonadina. *Collodictyon* emphasizes the difficulties arising in establishing the distinctness of the groups Pantostomatina, Protomonadina, Distomatineae and Polymastigina, especially when the nuclear phenomena are considered.

As to the location of the mouth, *Collodictyon* seems to have all of its body somewhat metabolic, the anterior end alone being comparatively constant in shape, but the function of ingesting food is localized in the sulcus, about one-fourth of the surface of the organism, assisted materially by the posterior part of the body. Thus, in this feature, *Collodictyon* seems to lie midway between the Pantostomatina and the Protomonadina or Polymastigina. If the primitive phylogenetic type be regarded as a polarized flagellate, with a surface entirely

amoeboid, then the sulcus of *Collodictyon* may be regarded as a vestigial character, a restricted surface area retained from a *Rhizomastigina* type. *Collodictyon* would thus be closely related to the rhizopods, not far removed from the ancestral type from which the latter diverged from the evolving flagellates. By such an interpretation it would naturally be considered an organism of a generalized or possibly a primitive type. If, however, the primitive phylogenetic type be regarded as a polarized flagellate with a non-amoeboid surface, then the sulcus of *Collodictyon* must be regarded as a highly specialized cytostome.

Among the specific and generic characters of *Collodictyon* which may be called diagnostic, I find:

1. The number of flagella is four. Carter erred in this.

2. I find no contracting vacuole (I am loath to say that there is none). Carter (1865) described but did not figure "contracting vesicles;" Kent (1880–82, p. 308), with seeming authority from Carter, denied that there were any; "such an open vacuolar character of the parenchyma would seem to obviate the necessity for a contractile vesicle, the presence of which structure Mr. Carter was unable to detect." Stein (1878–83) described and figured one in the anterior end near the nucleus; Francé (1899) with difficulty found one in the anterior end and timed its pulsation at about every forty seconds; Klebs (1893) found one in the posterior end in the *Tetramitus* he described as "*sulcatus.*"

3. The flagella are equal in length, being as long or slightly longer than the body. This possibly was the basis of Kleb's mistaken identity.

4. The sulcal region is a modified surface area and may be regarded as a cytostome. The classification of Delage and Hérouard (1895), Lankester (1909), and Calkins (1909) involves this in their Tribe Monostomatina.

5. There is an extranuclear centrosome just at the point where the rhizoplasts enter the nuclear membrane. It is, therefore, outside of, but connected with, the blepharoplast. In mitosis a paradesmose is formed between the dividing centrosomes. These characters establish polymastigote affinities.

6. Blepharoplast (so defined as not to include the division center) consists of two basal granules embedded in a chromatoidal matrix or surrounded by a granular archoplasm. The basal granules are connected to the nucleus by faint rhizoplasts.

7. In mitosis the nuclear membrane is persistent.

8. There is formed a perfect spindle of achromatic mantle-fibers with at least seven, probably eight, chromosomes and an equatorial plate.

9. Life cycle so far discovered is simple, binary fission by longitudinal division being the only method of reproduction known.

Collodictyon is typically an animal of simple organization. Its phylogenetic stem grows out of the unknown past. It is very close to the stem from which the Rhizopoda and Mastigophora branch, having much in common with each. That *Collodictyon* is one of the simplest and most primitive of the Polymastigina, there can be no doubt. With the free living members of the genus *Tetramitus* (not accepting *T. chilomonas* as such) it finds its nearest relatives. *Costia, Tetramitus* (saprophytic and parasitic), *Trichomastix, Polymastix,* and *Trichomonas* are derivatives of either the *Collodictyon* type or free living *Tetramitus.* Thus knowing possibly the simplest Tetramitidae, I am prone to regard this group as not so complex in its entirety as Francé (1899) would have us believe when he wished *Collodictyon* related to the Monadaceae rather than the "complex Tetramitaceae." Much of this complexity may be interpreted as the result of morphological changes resulting directly or indirectly from parasitism.

In *Collodictyon* the two basal granules with very faint rhizoplasts connected with the microkaryosome are not necessarily so highly differentiated, when it may be regarded as simply a slight advance over *Cercomonas* (Wenyon, 1910, text fig. 18), due to the multiplication of flagella, or a doubling of the flagella of a biflagellate type. *Collodictyon* is a primitive polymastigote.

Parasitism and Symbiosis

For some three months, January to March, 1916, the majority of all individuals of *Collodictyon* were filled with algae, which were identified as *Chlorella vulgaris.* Only at the period of longitudinal division and in moribund stages would they become free from these inclusions. The algae were arranged peripherally, just beneath the surface and it looked at times as though *Collodictyon* were hollow. Often when under observation these algae could be seen to form small nodules and pop out of the pellicle. These were seldom surrounded by a hyaline area indicating that they were being used for food, though at periods nearly all were so absorbed.

From the above similar and repeated observations I was forced to conclude that we have here a case of parasitism or possible symbiosis. Such a *Collodictyon* seldom engulfed food and seemed well nourished, as though by holophytic nutrition. For some months, in spite of daily observations, I never observed pseudopodia protruded from the sulcal region in such individuals, which condition is characteristic of the holozoic phase. If such be regarded as parasitism, *Collodictyon* must be regarded as the parasite, and *Chlorella* must provide the nourishment. Possibly it had best be regarded as a case of benign domestication, the by-products alone being used. To be truly a symbiotic relationship, *Chlorella* would have to be benefited, and I have not been able to determine that this was the case. I do know that it thrived fully as well, if not better, outside the organism.

The question of inclusions functioning in a parasitic or symbiotic relation, is not a new one. The "yellow cells" of Radiolaria is one of the most interesting as well as most disputed points, and one which is still far from being satisfactorily solved. They were first described by Huxley in *Thalassicolla,* and verified by Johannes Müller and Haeckel. Cienkowski (1871) strongly contended that they were parasitic algae. The discussion progressed with Richard Hertwig (1898, 1902), K. Brandt (1881, 1882, 1885), Entz (1882), and F. Keeble (1909), adhering with certain reservations or modifications to Cienkowski's interpretation. Müller had at first indicated the possibility that they were phases in the life cycle, but later gave up this conception. In 1909 Moroff and Stiasny contended that the yellow cells of *Acanthrometron pellucidum* were part of the developmental cycle; in 1910 Stiasny extended this interpretation to Sphaerozoa and Radiolaria generally. Such a view was not accepted without reservation by Minchin (1912); so the question stands, awaiting further and decisive evidence.

In *Collodictyon,* there can be no doubt that the inclusions are algae. They correspond with the free *Chlorella* of the aquarium. I did at first mistake them in observations upon unstained material to be elements of multiple fission, but was forced to give this up upon critical examination. Neither can it be a situation similar to *Euglena gracilis* (Zumstein, 1899), in which the typical chloroplast is lost and an *Astasia*-like phase results; though such evidence furnishes a most interesting possible analogy. That an ultimate lichenoid condition might result is a possibility and from such a relation *Collodictyon* might be transformed from a holozoic to an independent holophytic state.

The possibility of *Collodictyon* becoming ectoparasitic upon the gills or endoparasitic in the intestine is a very fertile field for speculalation and experiment. I have nearly always found *Collodictyon* when examining preparations brushed from the gills of goldfish of the aquarium. This may have been due to their normal abundance in the water. In attacking dinoflagellates, large *Euglena,* and other rapid, free-swimming organisms, *Collodictyon* may grasp the body of its prey in a death clasp with all four flagella. At times I have seen one flagellum free in such attacks. Normally, in attaching itself to the substrate all four flagella are spread out radially and its body may be drawn down close and tight or left suspended at a considerable height. In such a state it can revolve clockwise or counter-clockwise upon its major axis. We can hardly imagine *Collodictyon* modified directly into a *Costia*-like animal, though the differences are not so great. In *Costia* there are four flagella, two of which are long and used for fixation, two of which are short and used for wafting food to the cytostome. All four function in locomotion. In *Collodictyon,* the four flagella occur in two pairs with separate basal granules, but all are of equal length and undifferentiated in structure and function. When we consider its omnivorous propensities it seems possible that it could easily adapt itself to an ectoparasitic life, and possibly does so.

Its extreme delicacy of protoplasmic texture and tendency to rupture under slightly unfavorable conditions, makes it improbable that it may directly become an endoparasite, even in the rectal region of fishes. That it could not pass uninjured through the stomach and into the intestine, would be evident to all who could observe it closely. But its great variation in size with correlated reduction of cytoplasmic vacuoles, and the possible modification of the sulcal region into a cytostome similar to that in *Trichomonas,* or a cytostomal region of attachment, leads me to offer *Collodictyon* as a possible free-living ancestral type, or near relative of such at least, of these highly specialized genera.

Mitosis

Since the discovery of a method of cell division by Remak, the evidence of mitotic phenomena has accumulated rapidly. The early discussion, and late as well, centers about the universality and then the very existence of amitosis as a method of cell or nuclear division. To this mooted question my observations can add but little of decisive value. It is evident that *Collodictyon* presents still another example,

among flagellates, of mitosis. There is not sufficient evidence yet to generalize, but there is a probability, previously expressed by many, that with a fuller knowledge of flagellates, amitosis as a normal method of cell division in Protozoa will be reduced to a minimum. All work on flagellates and rhizopods in which amitosis has been recorded, should be reworked carefully and patiently, for the metaphase spindle is not a structure of long duration, very few instances being met with in the vast number of vegetative and karyokinetic individuals, and the various stages and figures of the prophase are readily capable of erroneous interpretation.

There are several important problems related to the differential division of the karyosome. In the first place it in no way involves the question of *amitosis* as a type of reproduction.

That there is here any phenomenon of chromatin reduction, homologous to *maturation,* must be regarded with equal skepticism. The phenomena of sex have been established for the Phytomonadina for years (Dobell, 1908). The nuclear details of the sexual process in this group are still hard to explain. In other flagellates sex phenomena have received little confirmation. Maturation, such as is regarded as necessary in sexual reproduction among higher plants and animals, has had little corroborative evidence among flagellates. Dobell (1908) worked out the life cycle of *Copromonas subtilis* and figures nuclear extrusion of chromatin in the form of two polar bodies. These are not described as the products of mitosis, however. On critical examination Dobell's work is not convincing. His evidence is inadequate and thus far has not been verified. Goldschmidt (1907) described similar phenomena for *Mastigella vitrea.* His observations are less satisfactory than Dobell's. Schaudinn (1904) in error extended the sexual process to *Trypanosoma noctuae.*

Nuclear extrusion of chromatin is, however, a common phemonenon in flagellates and protozoa generally. Work on *Trichomonas* (Kofoid and Swezy, 1915) reveals chromatin extrusion in each of these forms. Among *Amoeba* of the *limax* group (Alexeieff, 1911*b,* 1912*a,* 1912*b*), a similar extrusion occurs. But all of these extrusions as described, with the exception of *Copromonas,* seem to have no relation to maturation phenomena.

Collodictyon (pl. 10, figs. 29–36) presents a clear case of separation of chromatin from that which organizes for mitosis in the differential division of the karyosome (pl. 10, fig. 32). In one instance the macrokaryosome is seen in a state of division (pl. 10, fig. 33) very

comparable to the division of a polar body, but there is no adequate basis for any maturation process. Another phase (pl. 13, fig. 60) was found in which there are two nuclei in a partially constricted individual. This may be a late telophase, but in each nucleus can be seen a small chromatic body near the central karyosome. Furthermore there is a food vacuole containing a recently engulfed *Pandorina*. This is contrary to the rule. At the beginning of mitosis all food particles are extruded and this is the only instance in which it seems food has been engulfed before karyokinesis is completed. It is interesting, therefore, to contemplate the possibilities. It may be interpreted as follows: 1. A telophase phenomenon, presenting the anomalies of the small chromatic mass near the karyosome of unknown function, but probably metabolic, and the engulfed *Pandorina*. 2. A somatella of two cells or a suspended telophase which has actively begun to engulf food. 3. Conjugating individuals in which polar bodies are being extruded. Since conjugation has never been observed in living material and this is the only instance capable of such an interpretation, it seems improbable. The first alternative leaves much to be desired, since it gives no explanation of the exceptional phenomenon. This leaves the interpretation of a suspended telophase or a two-celled somatella as the most probable explanation. It can not be considered of much critical value until further verified and elucidated. We may, however, at least exclude the probability of maturation phenomena. In *Collodictyon* is found a beautiful illustration of the separation of excessive chromatin from the mass undergoing mitosis, thus freeing that body for its generative function. It presents little probability of sexual phenomena. If I may be allowed to surmise, acknowledging how illogical such a surmise is, all such maturation phenomena, recorded in the Flagellata, may be nothing more than the amitotic division of the karyosome, as is evident in *Collodictyon*, a freeing of the nucleus of excessive or surplus chromatin.

The *surface-volume ratio hypothesis* is not supported by the cell division of *Collodictyon*, since all sizes of cells are found undergoing division (pl. 11, figs. 40, 45, pl. 12, figs. 47, 51, 54). There is an apparent indifference to size.

The *theory of nucleao-cytoplasmic ratio* is much more difficult to prove or disprove positively. *Collodictyon* presents a double line of evidence: *First*, the differential division of the karyosome may be interpreted as either (*a*) a method of chromatin extrusion, such as has repeatedly been recorded for many, the majority of the Plasmodro-

mata; (*b*) simply the freeing of the karyosome of sufficient chromatin, which may here be regarded as passive and inert, to permit of unretarded kinetic activity on the part of the generative microkaryosome, or (*c*) both. The *second* line of evidence points toward a separation of the chromatin preliminary to a reorganization and growth of the same. It is necessary for the peripheral chromatin as well as that in the macrokaryosome to pass through solution, usually through the stage of a chromidial cloud as well, before entering into the prophase skein and the metaphase chromosomes whose organization takes place within the metabolic membrane. The chromatin upon the equatorial plate is much greater in amount than the mass of the original microkaryosome. Growth and organization have, therefore, definitely taken place. Subsequently, growth proceeds much more rapidly in the anaphase, during which time each daughter nucleus comes to contain almost if not fully as much chromatin as the original nucleus (pl. 12, figs. 52–55). This would indicate that cell division was not initiated by an unbalanced ratio and that the chromatin-cytoplasmic equilibrium may be simply a metabolic phenomenon, not at all related to initiating cell division. It would point, however, to the necessity of freeing the microkaryosome to insure its better kinetic activity and that a dividing nucleus seeks to be unretarded by surplus chromatin when organizing for division. *Collodictyon* thus presents evidence which tends to contradict the nucleo-cytoplasmic ratio theory.

The problem of dual chromatin is especially inviting. There are two types of chromatin in *Collodictyon,* differing clearly and unmistakably in behavior. Repeated efforts to get a differential stain for the macrokaryosome and microkaryosome, however, have not succeeded, and we have no evidence whatever of any chemical or physical difference between the chromatin of these structures. Observations upon the microkaryosome lead me to interpret that body as consisting of the generative chromatin. In its function at least, this chromatin is different from that of the separated macrokaryosome, which is both physiologically and morphologically passive. It required little speculation to conceive, in fact it is perhaps probable, that with differentiation in functioning, the chemical nature of the chromatin might be sufficiently altered by purely metabolic changes to produce differential staining of the two bodies. That such a differential staining has not been achieved, points emphatically to the fundamental similarity and chemical nature of all the chromatin of *Collodictyon. Collodictyon* seems to present an example among flagellates of trophochromatin and

idiochromatin. Such a distinction in this instance is, however, more physiological than morphological.

Conceived in such a category, *Collodictyon* may stand closely related to the parasitic flagellates containing a parabasal body (kinetonucleus of Hartmann). This would emphasize the fact that Hartmann's Binucleata is based upon a fundamentally physiological rather than a true morphological character. The macrokaryosome, through the changing metabolic equilibrium, brought about by change from a free living to a parasitic mode of life, might be preserved and persist as a result of purely chemical reaction, thus becoming the parabasal body. This possible origin of the parabasal body is purely theoretical. *Rhynochomonas* (Bělăr, 1916) and *Bodo caudatus* (Alexeieff, 1911*a*), as far as I know, are the only free living flagellates having any persistent character comparable to a parabasal body. The fact that the parabasal body is not present in all parasitic flagellates, nor constant in the life-history of some, would emphasize its dependence upon chemical nucleo-cytoplasmic equilibrium and its probable origin from a primitive condition such as is found in *Collodictyon.*

Werbitsky's work (1910), in which, by feeding host rats parafuchsin, tryparosin or oxazine, the parabasal body was dissolved and a strain of parasites obtained in which this body did not appear through several successive generations, may be similarly interpreted. Anything tending to counterbalance or upset the metabolic equilibrium of the cell would be expected so to affect a simple passive mass of chromatin which no longer functions in its original rôle, but persists as a surplus or reserve. Since chromatin seems to be the substance in which metabolism centers, the parabasal body would thus probably function as a kinetic reservoir for the motor activities of the cell. Evidence from *Collodictyon* emphasizes this interpretation of Kofoid and Swezy (1915).

Mitosis in Metazoa is variable in its details, but as found in the Protozoa it is variable in its fundamental features, so much so that it may be classified into categories. Little distinction was made in the types of mitosis until Nägler (1909, p. 46) designated what had previously been called amitosis in *Amoeba* and many Protozoa as "promitosis" in contrast to the type of mitosis as found in Metazoa and Metaphyta. "Fur die sogannte Amitose der Protozoen fuhrt man daher am besten eine neue Bezeichnung ein und definiert sie als eine Kernteilung, die weder ausgesprochene Mitose, noch Amitose ist und sich charakterisiert durch die Teilung eines Nucleocentrosoms, des Caryosoms. Ich schlage deshalb für diese Teilungsform den Namen

Promitose vor." Flemming (1882) suggested the terms, "amitosis" and "mitosis," and defined the former as a nuclear division without formation of chromosomes and a spindle, while in mitosis these are more or less evident. Nägler (1909, p. 46) thinks amitosis would be better characterized "durch die unregelmässige Durchschnürung des Kernes (Fragmentierung)." He concludes that an extreme instance of amitosis is not known in the Protozoa, instances so interpreted, with division of centriole within the karyosome and the apparent division of the whole chromatin mass, being more analogous to mitosis than amitosis.

Chatton (1910) distinguished three types of mitosis, which, as he characterized them, may be analyzed as follows:

Promitosis.—(Protokaryon type of nucleus, consisting of a fundamental mass of plastin, impregnated with chromatin, and containing a centriole).

1. Nuclear membrane is persistent and division is intranuclear.
2. Karyosome is the equivalent, morphologically and physiologically of the centrosome.
3. Equatorial plate (chromosomes?) organized from peripheral chromatin material.
4. Chromatin is not distributed equally by the nuclear mechanism.
5. Achromatic separation fibers are apparent when the karyosome divides.

Thus all essential elements and the primitive substances, except peripheral chromatin (which may be most important), are condensed within the karyosome. In higher forms of mitosis these tend to separate. "In proportion as the karyosome loses its plastin and chromatin elements, and becomes reduced to the centriole alone, so the primitive promitosis will approach more and more to the type of an ordinary mitosis" (Minchin, 1912, p. 110). This reduction of the karyosome may be either temporary, taking place only during the process of mitosis, or permanent, as is characteristic of higher types of mitosis.

Mesomitosis.—

1. Nuclear membrane persistent and division intranuclear.
2. Centriole, more or less separated from the karyosome, rests within the nucleus.
3. Chromosomes derived from the karyosome.
4. Chromosomes (equatorial plate) organized upon a spindle.
5. Plastin is reduced or disappears.

Metamitosis.—

1. Nuclear membrane disappears during process of mitosis, the mitotic figure resting in the cytoplasm.
2. Centriole, separate from the karyosome, may be intranuclear (as in *Pelomyxa*), but is generally extranuclear.
3. Evident chromosomes splitting longitudinally upon an equatorial plate.
4. With the zones of differentiated, surrounding cytoplasm the centriole forms the centrosome. Polar asters are usually present.

Chatton thus emphasized or summarized three categories:

1. Division center within the karyosome (nuclear membrane persistent, chromosomes organized from peripheral chromatin).
2. Division center outside the karyosome, but within the nucleus (nuclear membrane persistent, the karyosome organizing the equatorial plate).
3. Division center extranuclear (nuclear membrane disappears, chromosomes organized from nuclear chromatin).

Collodictyon falls into none of these categories. This system is based upon *Amoeba* and can not be extended without modification to include the typical polymastigote type of mitosis, in which the nuclear membrane is persistent and the division center extranuclear. Analysis of the mitotic phenomena of *Collodictyon* may be correlated and summarized here.

1, The unequal constriction or differential division of the karyosome into microkaryosomes and macrokaryosomes, which differ at least in behavior. The microkaryosome organizes directly into the segmenting skein. The macrokaryosome is: (*a*) extruded from the nucleus and absorbed into the cytoplasm, producing an extranuclear chromidial cloud; or (*b*) distributed as a unit to one of the daughter nuclei; or (*c*) is absorbed in the intranuclear cloud and probably is involved in chromosome organization. These two derivatives seem to offer good examples of so-called trophochromatin and idiochromatin.

2. The presence of an extranuclear centrosome. Intranuclear chromatin cloud.

3. The presence of a paradesmose.

4. Nuclear membrane persistent.

5. Successive separation of promitotic segmented skein. Indication of a precocious splitting of the peripheral chromatin granules of the nucleus. Evident precocious splitting of final segmented skein in prophase.

6. A well defined intranuclear spindle (of mantle-fibers). No extranuclear astral rays.

7. Definite chromosomes, organized upon equatorial plate, probably derived from chromatin of the microkaryosome and the intranuclear chromatin cloud formed by the going into solution of the peripheral chromatin granules and probably the macrokaryosome.

8. Apparent transverse splitting of the chromosome in metaphase.

That it is impossible to use Chatton's categories, unmodified, will inevitably be concluded from only a slight survey of protozoan mitosis. If emphasis be laid upon the division-center, accepting this as the

homologue of centriole, Binnenkörper and centrosome, and other characters be excluded, then the classification may be all-inclusive. But whenever other characters are correlated, exceptions or connecting links become as common or more so than the rule, and only arbitrary progress is made.

Alexeieff's "Systematization de la mitose dite 'primitive,' " (1913) into five types—promitose, haplomitose, mesomitose, paramitose, and panmitose—each subdivided into two subtypes, is the best possible illustration to what extremes one will be led who attempts to bring order out of the chaos of protozoan mitoses. With this elaborate schedule, there are many misfits and the exceptions become the rule or the logical connecting links. *Euglena* (Tschenzoff, 1916) fits into none of the categories. *Collodictyon* fails to conform to any of his categories, the centrosome being extranuclear and the nuclear membrane persistent. Furthermore, the chromosomes form partly from the chromatin of the microkarosome, partly it seems from the macrokaryosome and partly from peripheral chromatin granules, just as in panmitose. Thus it, too, is a conspicuous exception and emphasizes the arbitrariness of such attempts at elaborate classification.

Alexeieff (1913) attached little importance to the presence or absence of an equatorial plate, as a basis for classifying mitoses. His reasons assigned are very good and can be referred to by all interested. He emphasized the "centriole theory" and distinguished three types of mitotic figures as they possess (1) polar bodies, (2) centrioles, or (3) neither polar bodies nor centrioles. He suggested that polar bodies, reduced, might be homologous to centrioles, but then, with fine-spun distinctions, claimed that such were only present in mesomitosis and rheomitosis. What he regarded as the "pseudo-polar bodies" of haplomitosis, not being siderophile, could not be homologized with centrosomes by him; he, therefore, concluded that haplomitosis was very primitive and "particuliere." Hartmann (1911), Nägler (1909), and Chatton (1910) considered centrioles very general in Protozoan nuclei; Dangeard (1901), Alexeieff (1913), and Gläser (1912) considered them very rare.

Calkins (1903) suggested the constitution and rôle which chromatin plays in division as a basis for classification of the types of mitosis. Tschenzoff (1916) expressed the same suggestion, having failed to find a division center in *Euglena,* except the nucleolo-centrosome, the Binnenkörper not initiating cell division, but persisting much as a nucleolus of metozoa.

Chatton (1910) attached much importance to the absence, persistence, or disappearance of the nuclear membrane. Alexeieff (1913, p. 357) said, "Je ne puis pas portager l'opinion que la persistance ou la disparition de la membrane nucléaire a quelque importance." After citing *Centropyxis aculeata, Octomitus intestinalis,* where the nuclear membrane persists through all phases of mitosis, and *Hexamitus intestinalis* in which the nuclear membrane disappears, Alexeieff (1908) concluded: "En effet dans beaucoup de cas il est trés malaisé de décider si la membrane nucleaire a disparu complément, ou si elle est seulement amincie; souvent il n'y a q'une separation physique entre le cytoplasme et le suc nucleaire (comme entre deux liquides immiscibles) et l'image cytologique peut être dans ce cas difficile á interpréter."

Calkins in 1898 and in later works describes *Tetramitus chilomonas* as an example of a non-nucleated flagellate or rather of a distributed nucleus. He likewise (1899) considered *Chilomonos paramoecium* to have no nuclear membrane. Kepner and Edwards (1916) prove this latter to be incorrect. I came to the same conclusion from observations of material put up in 1916. I am, therefore, skeptical of the accuracy of Calkin's description of *Tetramitus chilomonas.* This form, so far as I can judge, is not a true *Tetramitus* but of a typical *Chilomonas* structure. The granules of the cell may or may not be chromidia. Distributed granules of some character (idiochromatin or paramylum) are characteristic of the genus *Chilomonas,* The division center would thus be considered either within the nucleus or the karyosome. It needs correction, verification, or elucidation.

The nature of the division center in flagellates is not well understood. Prowazek (1903) classifies flagellates with regard to this division center and his work was accepted with slight additions by Dobell (1908). Chatton (1910) classified primitive mitoses in *Amoeba,* basing his system upon the relationship of the division center to the nucleus and karyosome. Alexeieff (1913) gave a most elaborate system of the primitive mitoses, so elaborate, in fact, that it is of little service. Most references in recent years have reverted to the simpler system of Chatton (1910). In all of these the relationship of the division center to the nuclear chromatin has been accepted as the basis of differentiation. Calkins (1903) based his system upon the behavior of the chromatin. He (1899), however, based his conception of the evolution of metazoan mitoses upon the extranuclear division center of *Noctiluca.*

Hartmann (1910) in his new group Binucleata adopted the conception of an extranuclear division center located in the blepharoplast. This seems to hold good for many polymastigotes. There is always present a paradesmose, which is probably comparable to the centrodesmose of rhizopods or the "Binnenkörper" of free-living flagellates.

Kinetic Membrane

The phenomenon of a membrane organizing around the microkaryosome, commensurate with the inner boundary of the hyaline area, and expanding progressively during the prophase until it approaches and becomes identified, identical except for the part where the macrokaryosome rests, with the nuclear membrane, as is found in *Collodictyon,* so far as I know, has been recorded in no other instance. Achromatic radiations from the karyosome through the surrounding hyaline area are found in the nuclei of some amoebas (usually described as characteristic of protokaryon type of nucleus). These have been interpreted as being related more or less closely to chromosome formation. Sutton (1900) discovered what he designated "chromosome vesicles," surrounding the organizing chromosomes in an orthopteran insect. Carothers (1915) finds the same in the nuclei of *Culex* and modifies the term to "chromomere vesicle." Wenrich (1916) interprets these vesicles as expanded granules.

In *Collodictyon* the nuclear membrane is not one of the most evident features of the resting nucleus, but undoubtedly it is both present and persistent. With the separation of the microkaryosome and the beginning of organization, a second membrane, very faint but evident, is formed immediately around the microkaryosome, and progressively expands. Just around this membrane is a progressively expanding hyaline area, and inside is a more or less homogeneous clouded area filling up the entire intramembranous space, in which can also be distinguished the organizing microkaryosome, segmented skein and spireme. No such membrane surrounds the macrokaryosome and there is no evidence that any kinetic activity is present in or around this mass of inert chromatin.

R. S. Lillie (1902, p. 420) in discussing the oxidative processes of the cell-nucleus, concludes that "in many tissues the nucleus is the chief agency in the intracellular activation of oxygen . . . The active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm." The nucleus in much recent literature is regarded as the kinetic or metabolic center of cell

activity. It is usually concluded, on account of this, that the chromatin is the substance upon which metabolism is dependent. *Collodictyon* presents evidence bearing on this point. The macrokaryosome, consisting of separated chromatin, appears wholly passive during the prophase. The microkaryosome, on the other hand, consisting of chromatin which organizes the skein, is the center of great activity. The radius of this activity is bounded by the metabolic membrane. If this activity be regarded as due to the entering into solution of peripheral chromatin and related nuclear substance, which must pass into the interior, the so-called membrane must be either merely a static atomic equilibrium zone, through which outer and inner activities are counterbalanced, or it must be a definite membrane organized by great pressure from within and without, through which, by the process of osmosis, the peripheral solution passes into the inner sphere of organization, where the pressure is relieved and less than on the outside, by the condensation and precipitation of chromatin on the organizing skein. The latter seems the more plausible interpretation. In this light the generative chromatin is the kinetic factor, at least in mitosis probably in protozoan metabolism. The free chromatin is non-active and non-kinetic, acting in a purely passive way in the prophase. Since no food is engulfed and all inclusions are extruded, nutritive processes seem suspended in *Collodictyon* during mitosis. Oxidative (katabolic) processes would naturally be at their height, and better subject to analysis since they are here separate from the anabolic. Since the chromatin of the macrokaryosome shows no kinetic phenomena, chromatin as such may be eliminated as the center of "activation of oxygen." But since this oxidative katabolism is a part of nuclear activity, it is possible that it may be performed by the generative chromatin in *Collodictyon,* possibly in other protozoans; while anabolic or constructive processes of intussusception may find their center in chromatin.

There seems little doubt that the metabolic membrane found in *Collodictyon* is related in some way to the chromosome vesicles of the Metazoa, especially the radiations from the karyosome in a protokaryon type of nucleus. By its unity and simplicity I judge it to be more primitive. The chromosome vesicles fuse as mitosis progresses in higher forms; in *Collodictyon* it begins as a unit and ends in becoming practically continuous with the nuclear membrane. It may not be too imaginative to regard the nuclear membrane itself, with its peculiar phenomena of persistence or disappearance in mitosis.

as related in some way to this kinetic membrane, either in origin or in underlying causes.

The substance of the microkaryosome is quantitatively smaller than the chromatin of the metaphase chromosomes (equatorial plate). These latter seem undoubtedly to be derived from both peripheral and karyosome chromatin. The most probable way in which peripheral chromatin can get upon the skein or into the metabolic membrane, is by entering into solution and being again deposited on the inner achromatic framework, within the membrane. This would eliminate any interpretation of the continuity of all of the substance of chromosomes in *Collodictyon*. Much has been written of late concerning the individuality of chromosomes and this is regarded by many as fundamental to any mechanism of Mendelian inheritance, especially as interpreted by Jannsen and Morgan in their "chiasma-type theory" or "theory of crossing over." Wenrich (1916) presents probably the best morphological evidence of the continuity of the chromosomes, so far recorded. He, however, does not claim to present observable proof of such continuity, but finds in his observations and correlations, together with reasonable conclusions from hybridization and other experiments, that the evidence is greatly in favor of such continuity. Moenkhaus' (1904) conclusion from his hybridization experiments is typical: "If from such a nucleus, two kinds of chromosomes again emerge, it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that unit persists."

In *Collodictyon* the chromatin cycle consists of an apparently homogeneous karyosome, separation and possible elimination of major part, an apparent solution phase, prophase segmented spireme with terminal knobs, metaphase chromosomes, anaphase synizesis and growth, followed by a typical spireme of small nodular granules, which later unite into irregular masses, from which the daughter karyosome is reorganized.

Inheritance in Binary Fission

In sexual plants and animals the hereditary substance can be localized in the germ cells, though all reproduction is not by means of such germ plasm. In flagellates, reproducing alone by the method of simple binary fission, and in which sex is unknown, the problem of heredity becomes more complex, instead of simpler of analysis.

Were the problem solved for higher organisms, those conclusions

might be extended to the majority of the simpler Protista, but not necessarily to all. For in this group nature has her experimental laboratory, and we would expect to find discards. We may well believe that the mechanism found in sexual plants and animals is an extremely modified and specialized adaptation of a much simpler, more fundamental, but thoroughly satisfactory type, characteristic of most binary fission as found today. So much at least we may assume.

Parthenogenesis might be pointed to as a reversion to such a primitive type and such may be the case; but a study of this phenomenon points rather to its being a still further specialization and adaptation, based upon advanced sexual phenomena or their suspension. If such be the case, we need not look here for the simpler type mechanism of inheritance characteristic of binary fission. Some do regard it as such, however, and such an interpretation naturally leads to the conclusion that sex is a universal phenomenon. Minchin (1912, p. 130) makes the generalization that sex is "of universal occurrence in all truly cellular organisms." This attitude does not seem to accord, nor can it be satisfactorily harmonized with facts as understood today. Coulter (1914) would refute such a view, at least in algae. In the simpler flagellates a satisfactory example of syngamy has yet to be found. Dobell's (1908) life cycle of *Copromonas* has not been verified. What he figures as maturation phenomena may be well explained by comparing his figures with the differential division of the karyosome in *Collodictyon*. Still variation and evolution are characteristic of flagellates. In fact, it is back to the flagellates that the origin of higher plants and animals is traced by the large majority of biologists.

It is little that *Collodictyon* adds to this much discussed subject. There is a mitotic figure, a mechanism which may well be interpreted as a distributor of hereditary characters. Chromosomes are present. The actual number of these in *Collodictyon,* as in most Protozoa, is very difficult to determine. They seem composed for the most part, the most evident part, of chromatin, probably upon an achromatic center or skeletal structure. Such achromatic elements must not be confused with "interzonal or connecting fibers," exposed by the diverging chromosomes (pl. 12, figs. 51–53). The chromosomes in the metaphase split transversely. Such a transverse division is capable of interpretation as a longitudinal split in two ways. Either the chromosomes split longitudinally, separate at one end and finally pull apart at the other end, or, during the precocious splitting of the spireme and final prophase the number of chromosomes is doubled

(pl. 11, fig. 47), these being fused end to end into half the number on the equatorial plate. It is, then, these doubled chromosomes which separate transversely in the metaphase. A third alternative is that the chromosomes do split transversely, the inheritable characters being usually halved physiochemically but not necessarily according to the chiasma-type hypothesis.

In behavior, at least, there are two kinds of chromatin. That of the macrokaryosome is largely passive, that of the microkaryosome is either active or activated by a close association with the division center. The former may possibly be analogous to the macronucleus of ciliates and the parabasal body of parasitic flagellates; the latter to the micronucleus of ciliates and the typical nucleus of flagellates. The distinction of trophochromatin and idiochromatin might be applied here as well as in any of the typical usually cited instances. The chromatin may be all of like character, its behavior being determined in all cases by associated elements. Its close association with achromatic elements and its inclusion in all chromosomes is, therefore, essential.

GENERAL SUMMARY

1. The first evidence of mitosis is an unequal constriction and differential division of the primary karyosome of the vesicular nucleus into a macrokaryosome and a microkaryosome, the latter alone functioning directly in the formation of the prophase skein.

2. The skein originates by the successive segmentation of the microkaryosome, resulting in two crescents and four terminal knobs.

3. These crescents split longitudinally, producing presumably eight terminal knobs which are the elements at least of chromosomes. It is possible that one of the terminal knobs fails to split. In this case the number of chromosomes would be seven, which coincides with the best count so far made.

4. Coincident with the beginning of the segmenting skein, there is organized around the microkaryosome a kinetic membrane which expands until it becomes apparently commensurate with the nuclear membrane.

5. In the final prophase there is a precocious splitting of the segmented skein, in which the number of terminal chromatin masses is doubled, and all are organized in an equatorial belt. These are probably fused in telosynapsis.

6. The spindle is intranuclear. There are seven or eight chromosomes which part transversely at the metaphase.

7. Growth of chromatin is very rapid in the anaphase. As the chromatin passes to the poles of the spindle, a distinct granular spireme is organized.

8. *Collodictyon* conforms to no category in the classification of mitoses of either Chatton (1910) or Alexeieff (1913). Its nuclear membrane is persistent and its centrosome extranuclear. A typical paradesmose is present. The chromosomes are organized from both peripheral chromatin and the karyosome.

9. *Collodictyon* is one of the simplest of the Polymastigotes, both in morphology and mitotic phenomena.

10. The blepharoplast consists of two basal granules, surrounded by a granular archoplasm. In the middle of the prophase these granules separate and divide into four, thus producing a double blepharoplast for each daughter cell. The flagella either split or grow out anew. The rhizoplasts split longitudinally, being doubled about the time the basal granules separate.

11. Division finally takes place by a longitudinal constriction along the sulcus.

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EXPLANATION OF PLATES

Figures in plates 7 and 14 are diagrammatic, those of plate 7 being based upon observations of living and stained material, plate 14 being sketched from camera lucida drawings of accompanying plates. Figures in plates 8 to 13 are camera lucida sketches. All figures except plate 13, figure 62, are of *Collodictyon triciliatum* Carter, killed in hot Schaudinn's fluid and stained in Heidenhain's aqueous iron-alum haematoxylin, unless otherwise stated. × 1700.

PLATE 7

Fig. 1. Laterosulcal view, showing vacuolated cytoplasm, sulcus, vesicular nucleus with karyosome and peripheral chromatin, blepharoplast of two basal granules surrounded by granular archoplasm, four flagella, and rhizoplast.

Fig. 2. Absulcal view, showing bifurcated posterior end, nucleus, blepharoplast, and flagella.

Fig. 3. Sulcal view, showing median sulcus, posterior bifurcation, nucleus, blepharoplast and flagella.

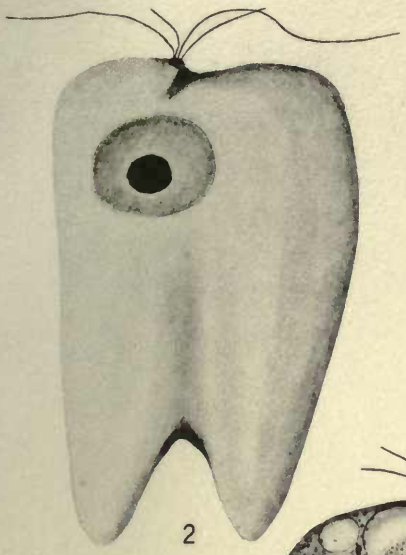
Fig. 4. Anterior view, showing sulcus, nucleus, blepharoplast, rhizoplast, and four flagella.

Fig. 5. Sulcal view of truncated posterior end; two cusps.

Fig. 6. Sulcal view of truncated posterior end; three cusps.

Fig. 7. Laterosulcal view of truncated posterior end; four cusps.

Fig. 8. Laterosulcal view of truncated posterior end; five cusps.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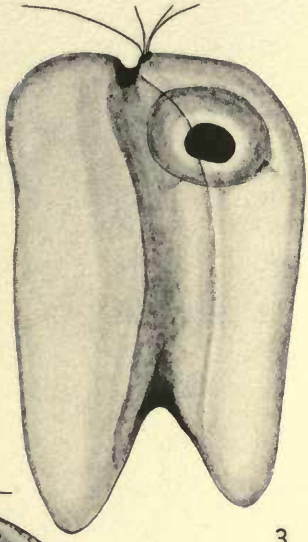
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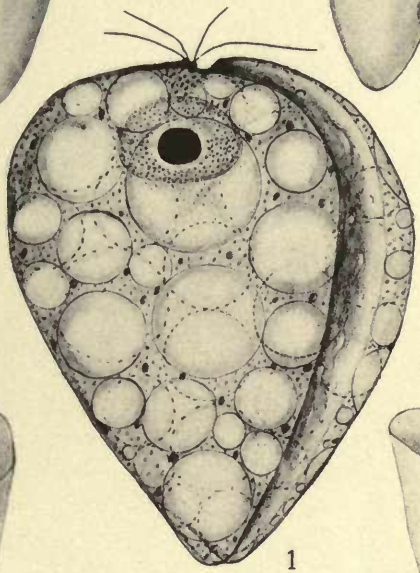
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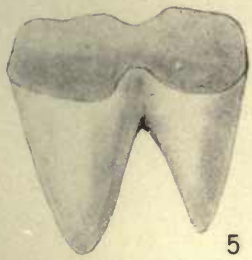
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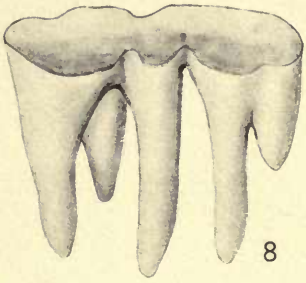
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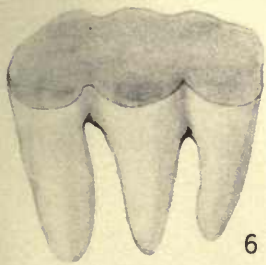
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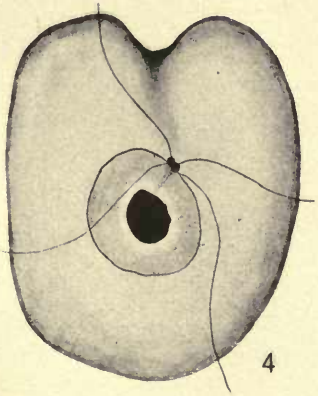
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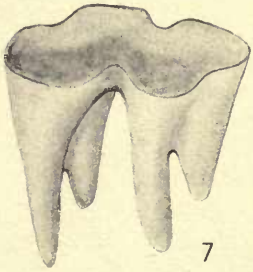
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PLATE 8

Figures 9 to 18, showing variations in size and shape.

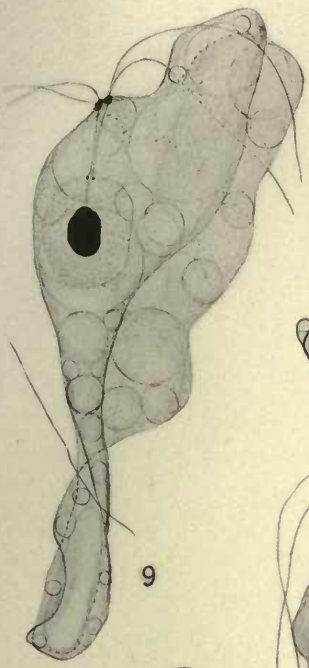
Figs. 9 and 16, from material killed in picro-mercuric, stained in Bordeaux R, aqueous iron haematoxylin.

Figs. 10, 13, and 18, from material killed in strong Flemming, stained in aqueous iron-alum haematoxylin.

Figs. 11, 14, and 15, from material killed in hot Schaudinn's fluid, stained in alcoholic iron-alum haematoxylin.

Fig. 12, from material killed in picro-mercuric, stained in Mallory's connective tissue stain, modified.

Fig. 17, from material killed in picro-mercuric stained in phosphotungstic acid haematoxylin.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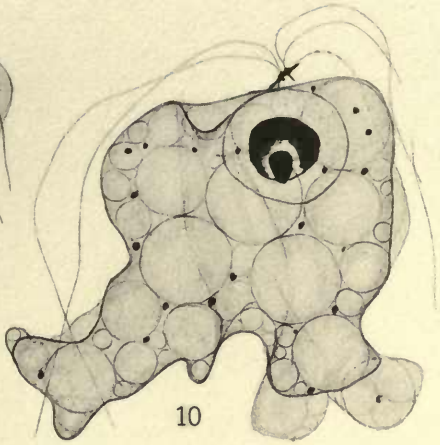
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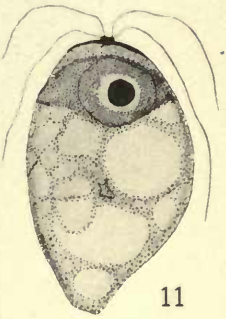
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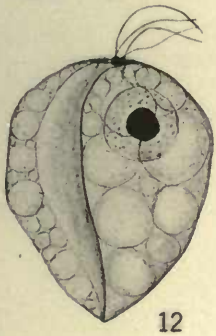
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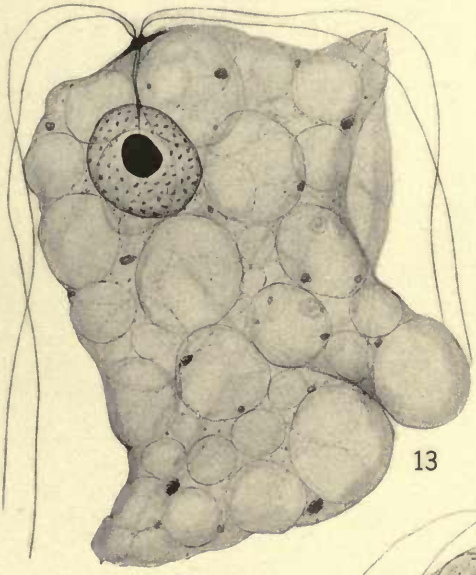
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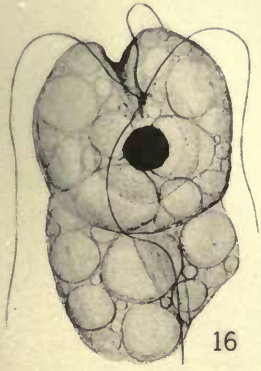
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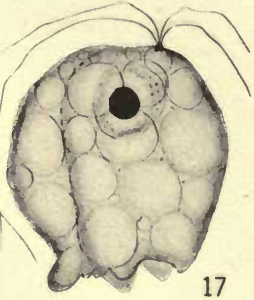
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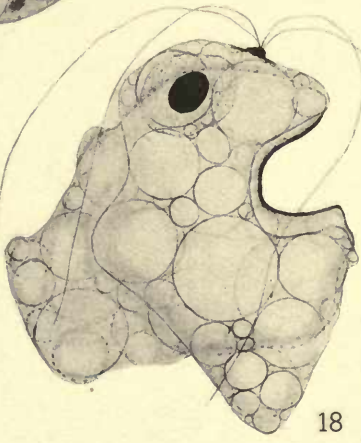
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PLATE 9

Figures 19 to 27, showing food inclusions, fig. 19 killed in hot Schaudinn's fluid, fig. 26 killed in strong Flemming's and stained in iron haematoxylin, and figs. 20–25 and 27 killed in strong Flemming's and stained in Bordeau R iron haematoxylin.

Fig. 19. *Ulothrix.*

Fig. 20. Two dinoflagellates, presumably *Peridinium.*

Fig. 21. Gelatinous capsule of *Pandorina* and *Scenedesmus.*

Fig. 22. *Scenedusmus.*

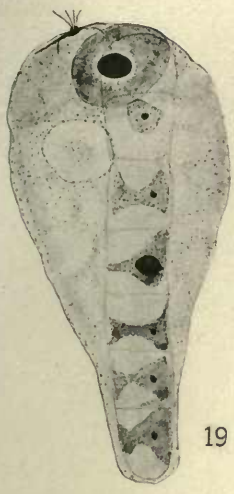
Fig. 23. *Pediastrum.*

Fig. 24. A diatom, presumably *Navicula.*

Fig. 25. A ciliate, presumably *Colpidium.*

Fig. 26. A diatom, presumably *Navicula.*

Fig. 27. *Pandorina.*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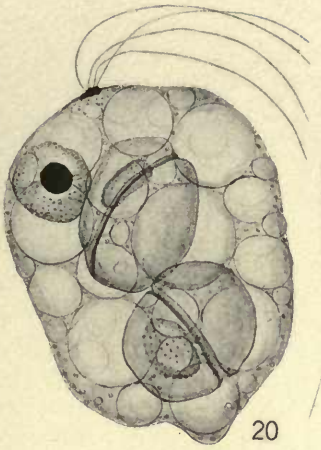
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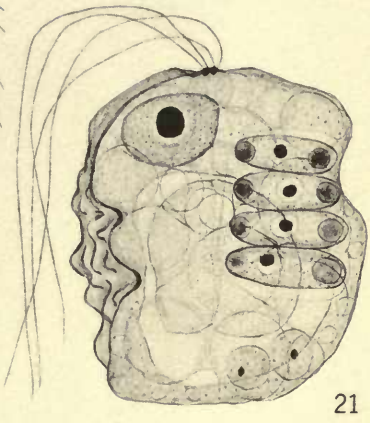
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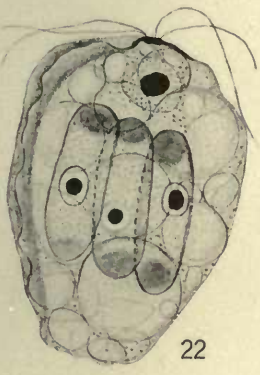
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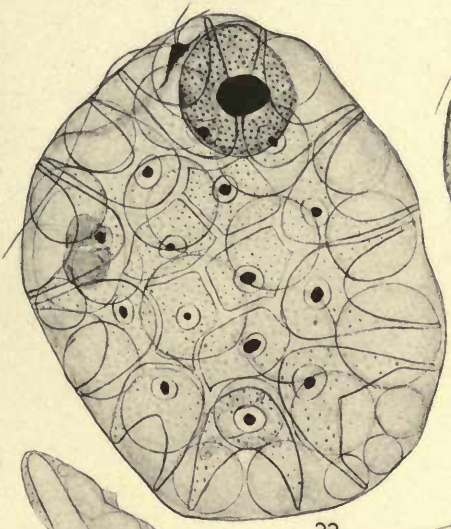
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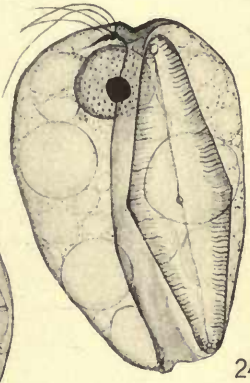
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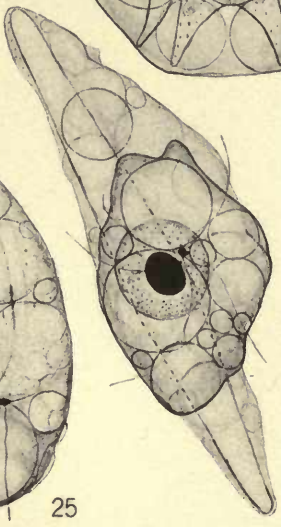
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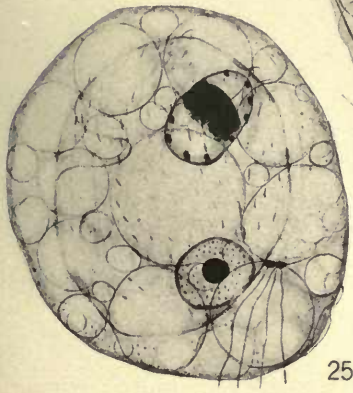
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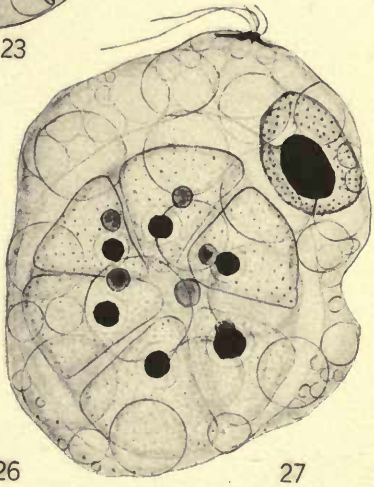
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PLATE 10

Prophase.

Fig. 28. Typical vesicular nucleus. Chromidial granules forming around the hyaline area.

Fig. 29. Unequal constriction of primary karyosome.

Fig. 30. Unequal constriction of primary karyosome. Organization of peripheral chromatin into granules connected by a slightly chromatic continuous fiber. Partial separation of basal granules.

Fig. 31. Differential division of primary karyosome into a macrokaryosome and a microkaryosome. Kinetic membrane surrounding the microkaryosome.

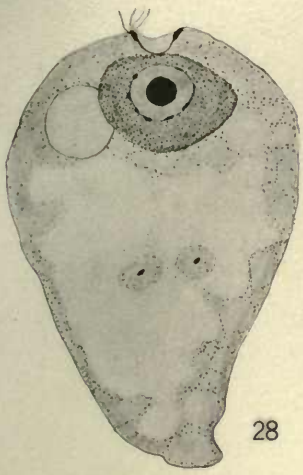
Fig. 32. The same as figure 31 with peripheral chromatin encrusted upon the nuclear membrane. Extranuclear chromidial cloud.

Fig. 33. The unequal constriction of the macrokaryosome. Note the granular organization of the microkaryosome.

Fig. 34. First signs of segmentation of microkaryosome.

Fig. 35. Segmenting microkaryosome with fibers connecting the two polar chromidial masses. Intranuclear chromidial cloud.

Fig. 36. The same as figure 35; nucleus elongated; peripheral chromatin granules; separation of basal granules, only four flagella.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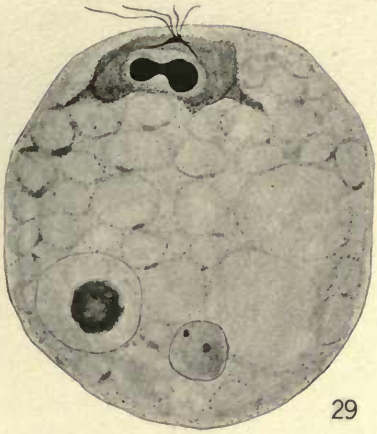
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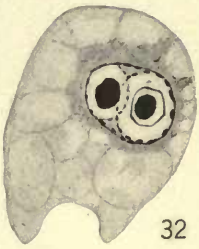
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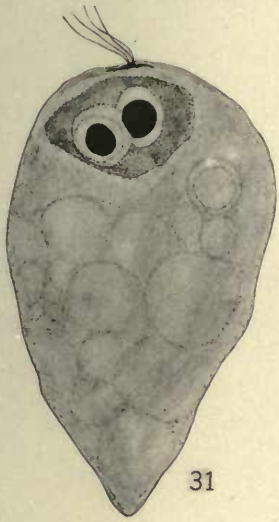
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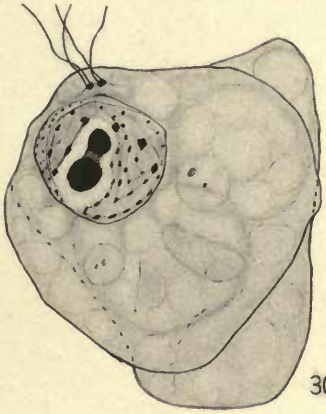
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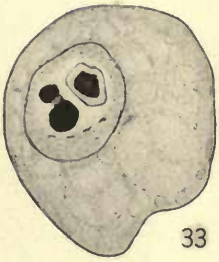
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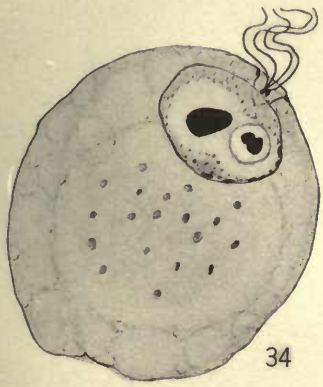
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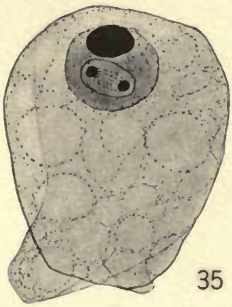
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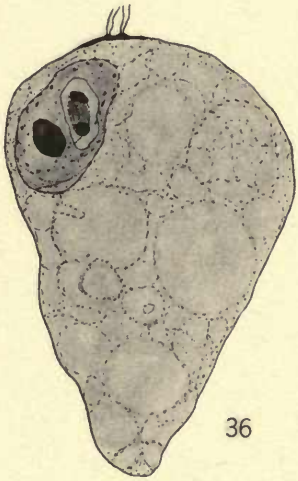
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PLATE 11

Prophase.

Fig. 37. Chromidial masses with connecting fibers in the segmenting skein. Chromidial cloud within the kinetic membrane. Macrokaryosome being pushed aside by the expanding kinetic membrane. Peripheral chromatin gathered just within the nuclear membrane.

Fig. 38. Macrokaryosome in a niche of the nuclear membrane. The segmenting skein within a precocious spindle formation.

Fig. 39. Further organization of the skein into the tripod stage.

Fig. 40. Tripod stage of segmenting spireme. Peripheral chromatin in larger granules. Further separation and initial division of basal granules, producing four basal granules and eight flagella, splitting of rhizoplast.

Fig. 40*a*. Another view of figure 40, showing the four basal granules.

Fig. 41. Segmenting skein in the form of a double crescent with four terminal knobs of chromatin. Two small granules which may be the division center. Chromidial cloud within the kinetic membrane, macrokaryosome passive.

Fig. 42. Same as figure 41.

Fig. 43. Disintegration of macrokaryosome. A moribund stage.

Fig. 44. Expanding kinetic membrane commensurate with the nuclear membrane. Macrokaryosome in a niche of the nuclear membrane. Some chromatin still encrusted upon the membrane. Rhizoplast evident.

Fig. 45. Longitudinal splitting of segmenting skein, producing seven or eight terminal knobs, in all probability the elements of the future chromosomes.

Fig. 46. Blepharoplasts separated. Precocious splitting of peripheral chromatin. A precocious equatorial plate with macrokaryosome apparently upon it.

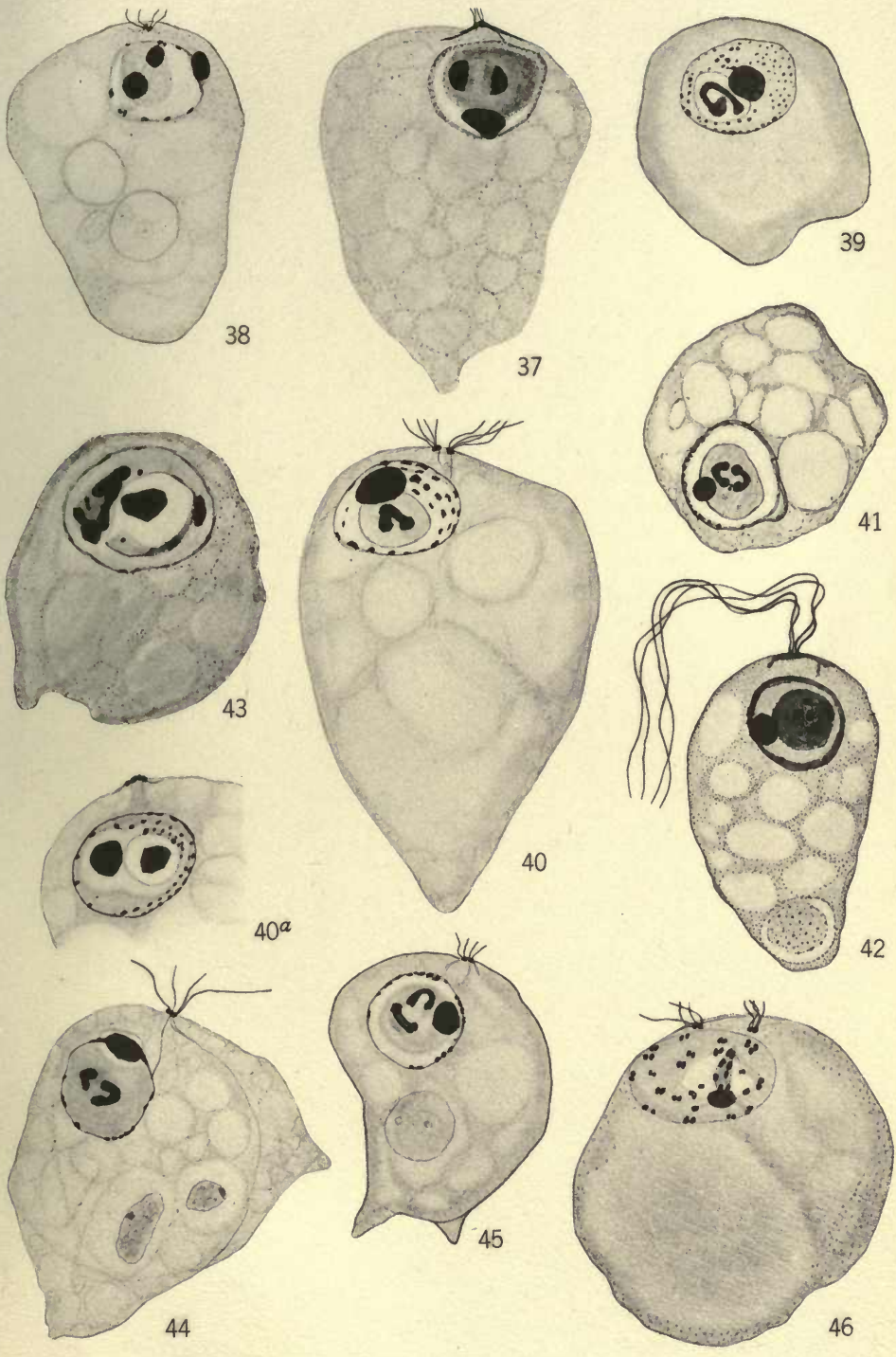
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PLATE 12

Metaphase, Anaphase and Telophase.

Fig. 47. Precocious splitting of the terminal knobs of the final segmentation skein, forming an equatorial belt of chromidial cloud in which are embedded eight paired chromatin masses. Final prophase.

Fig. 48. Metaphase equatorial plate. Macrokaryosome apparently a part of it. Two blepharoplasts of two basal granules each. Spindle oriented either in relation to the blepharoplasts or the major axis of the cell. Intranuclear chromidial cloud.

Fig. 49. Same as figure 48; chromatin granules lodged upon the spindle fibers. Clearing up of intranuclear chromidial cloud.

Fig. 50. Metaphase spindle. Seven chromosomes can be seen, one being only half so large as the others. Transverse splitting of all chromosomes except the small one. No chromatin within the nucleus except that upon the equatorial plate. No evidence of macrokaryosome, peripheral chromatin, or centrosome granules. From material killed in hot Schaudinn's fluid and stained in safranin, gentian-violet, orange G.

Fig. 51. Anaphase. Unequal amounts of chromatin passing to the daughter poles. The chromosomes are stuck together. Intranuclear chromidial cloud.

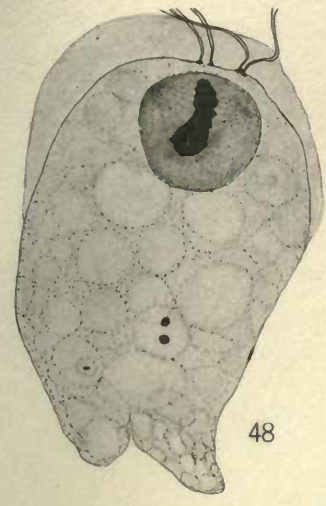
Fig. 51. Anaphase. Irregularly lobed chromatin masses collected at respective daughter poles. Separation fibers evident. Intranuclear chromidial cloud.

Fig. 53. Anaphase. Organization of daughter chromatin masses into linear skeins. Daughter rhizoplasts connecting the daughter blepharoplasts.

Fig. 54. Telophase. Daughter nuclei separated. Intra- and extranuclear chromidial cloud. Daughter blepharoplasts and related nuclei shifted to opposite sides of the sulcus.

Fig. 55. Telophase. Same as figure 54. Extranuclear chromidial cloud deepens.

Fig. 56. Chromatin has separated out into four large masses, an extra large mass being in one daughter cell. Daughter rhizoplasts heavy.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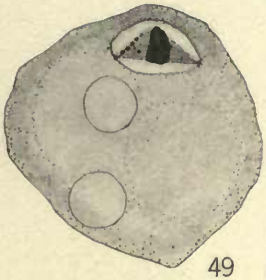
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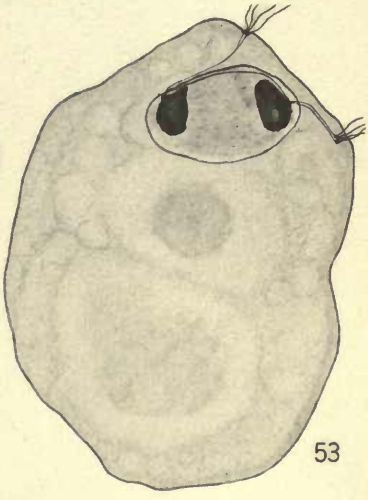
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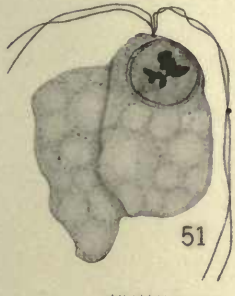
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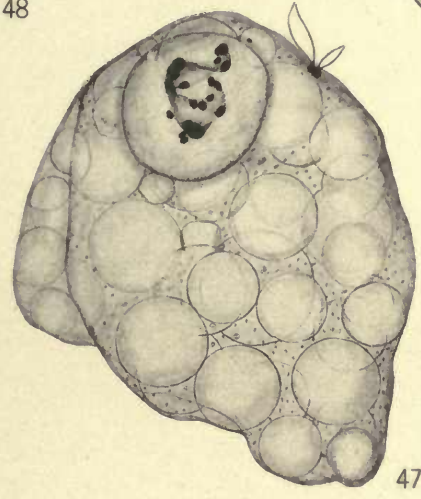
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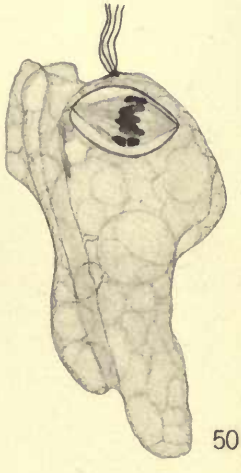
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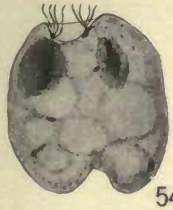
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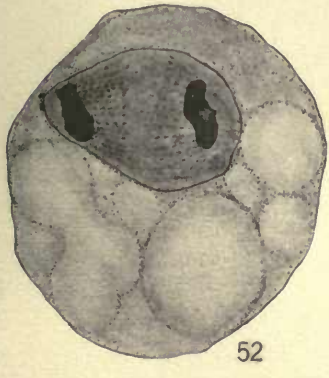
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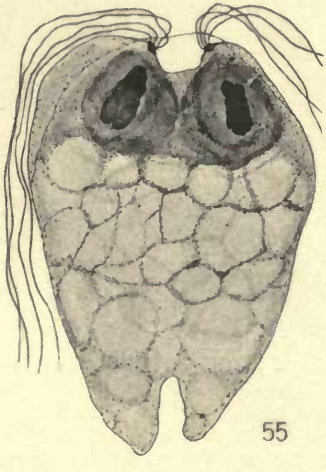
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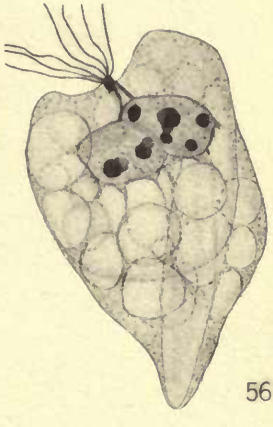
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PLATE 13

Fig. 57. Telophase. Cytoplasmic constriction along the sulcus. Blepharoplasts deeply stained. Rhizoplasts evident. Heavy extranuclear chromidial cloud. Chromatin further broken up into irregular masses.

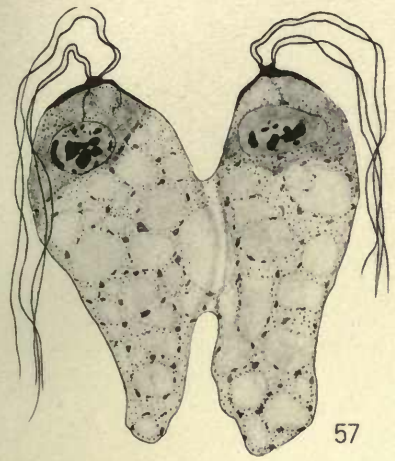
Fig. 58. Large vacuole in the cytoplasmic connective. Further dissociation of chromatin masses into granules which show the beginning of concentric organization.

Fig. 59. Reorganization of central karyosome and peripheral chromatin. From material killed in Flemming strong, and stained in Bordeaux R iron haematoxylin.

Fig. 60. Suspended telophase. Engulfed *Pandorina* in food vacuole. Karyosome and peripheral chromatin. Small chromatin mass outside the karyosome of unknown significance, possibly the division center. From material killed in Flemming strong, and stained in Bordeaux R iron haematoxylin.

Fig. 61. Individual just after fission is completed. Double rhizoplast extending into karyosome. Chromidial organization still evident in the karyosome.

Fig. 62. Somatella, probably of sixteen cells, of *Amoeba radiosa*. At first considered a *Collodictyon* cyst. Such may possibly be the case though reaction to the stain does not warrant such a conclusion. × 1900.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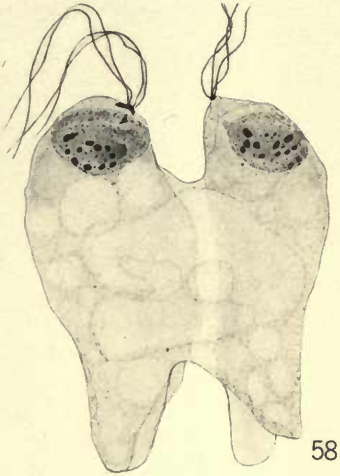
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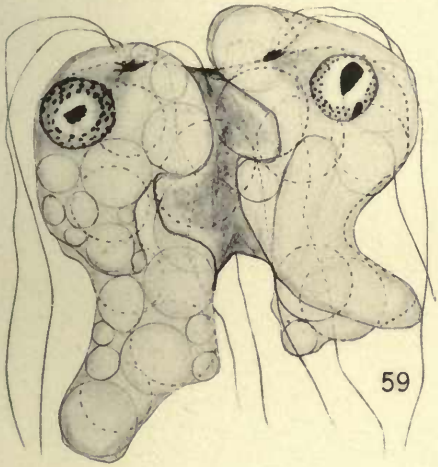
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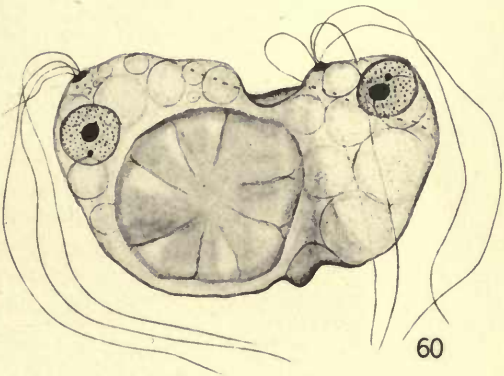
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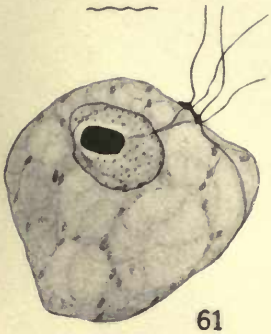
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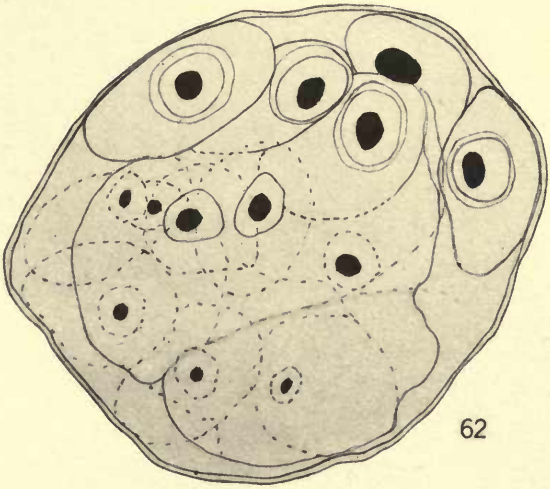

62

PLATE 14

Nuclear changes in binary fission in *Collodictyon*. Any differences between these figures and those of associated plates must be referred to the original camera lucida sketches for critical interpretation.

Figs. 65–76. Prophase phenomena.

Figs. 63–65. Stages of the resting nucleus.

Figs. 77–79. Metaphase.

Figs. 80–83. Anaphase.

Figs. 84–87. Telophase.

Figs. 66–67. Unequal constriction of primary karyosome.

Fig. 68–69. Differential division of the primary karyosome into a macrokaryosome and a microkaryosome. Organization of a kinetic membrane around the microkaryosome.

Figs. 69–73. The segmenting skein, with associated expansion of the kinetic membrane.

Fig. 71. Precocious spindle formation. Macrokaryosome in a niche of the nuclear membrane.

Fig. 72. Double crescent stage of segmenting skein showing four terminal knobs and a possible intranuclear division center dividing.

Fig. 73. Longitudinal splitting of crescents, producing seven or eight terminal knobs of chromatin which are the elements of the chromosomes.

Fig. 74. Precocious spliting of peripheral chromatin granules. Precocious equatorial plate formation with macrokaryosome upon it.

Fig. 75. Final prophase. Division of blepharoplasts with apparent splitting of flagella; separating centrosomes upon the nuclear membrane connected by a paradesmose.

Fig. 76. Precocious splitting of final stage of segmenting skein. Sixteen chromatin masses in a chromatin cloud in the form of an equatorial belt.

Fig. 77. Metaphase spindle. Macrokaryosome apparently a part of it. Intranuclear chromatin cloud.

Fig. 78. Metaphase spindle; centrosomes at poles of spindle and connected by paradesmose.

Fig. 79. Metaphase spindle showing seven chromosomes. Transverse parting of all chromosomes except one which is only half so large as the rest.

Fig. 80. Anaphase. Apparent unequal distribution of chromatin.

Fig. 81. Anaphase. Daughter chromatin masses organized into linear spiremes.

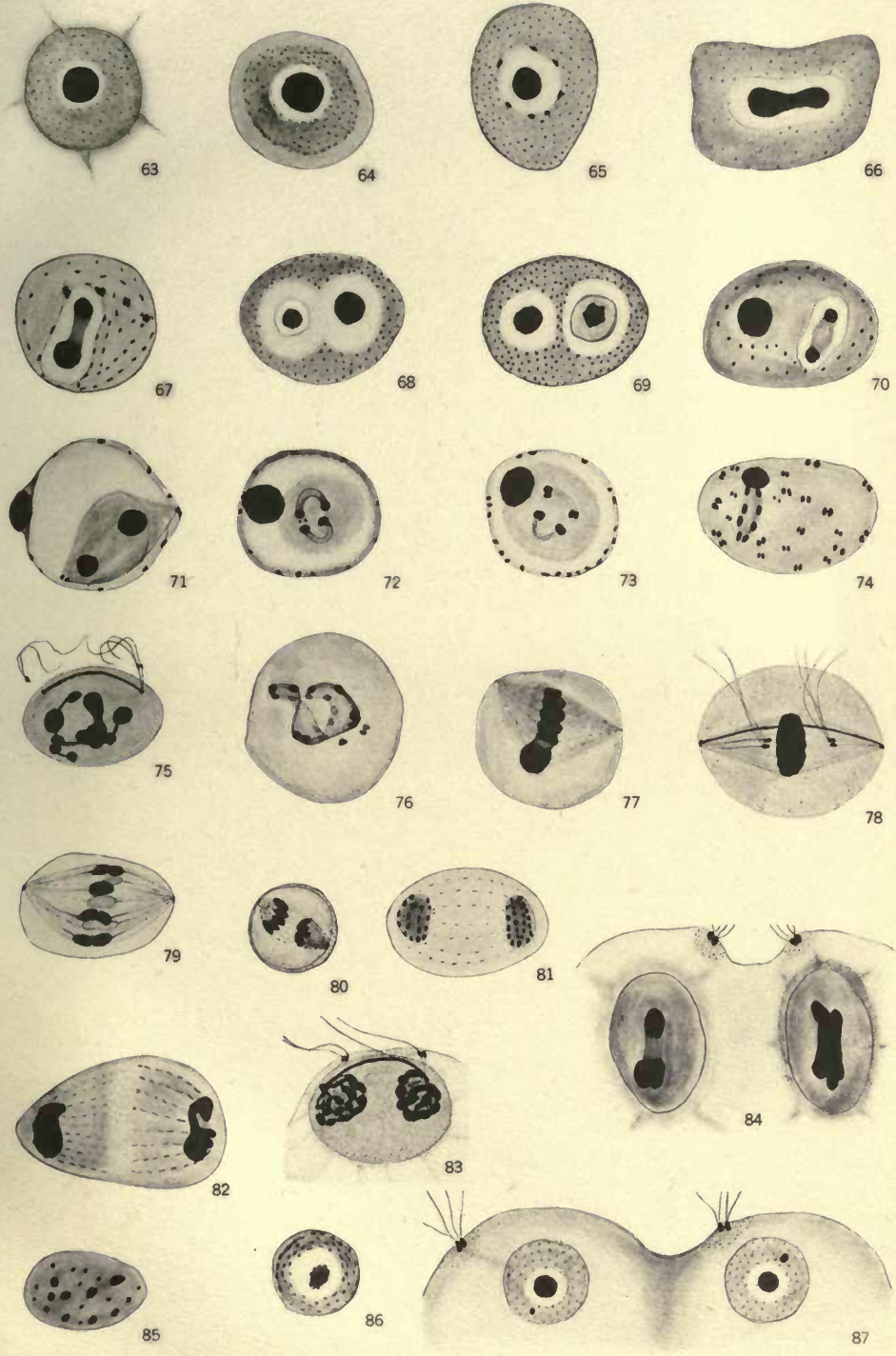
Fig. 82. Anaphase. Linear spireme of chromatin granules closely related to centrosomes, which are connected by paradesmose.

Fig. 83. Telophase. Complete separation of daughter nuclei. Extranuclear and intranuclear chromidial cloud.

Fig. 85. Distribution of chromatin as granules throughout nucleus.

Fig. 86. Reorganization of central karyosome with surrounding hyaline area, and peripheral chromatin.

Fig. 87. Suspended telophase. Vesicular nucleus with small chromatin mass of unknown significance near the periphery. Daughter blepharoplasts with double basal granules surrounded by granular archoplasm.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87

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Index, pp. 537-545.

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3. Description of Some New Species of *Polynoidae* from the Coast of California, by Christine Essenberg. Pp. 45-60, plates 2-3. October, 1917 ..... .20

4. New Species of *Amphinomidae* from the Pacific Coast, by Christine Essenberg. Pp. 61-74, plates 4-5. October, 1917 ..... .15

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2. The Pteropod *Desmopterus pacificus* (sp. nov.), by Christine Essenberg. Pp. 85–88, 2 figures in text. May, 1919 ..... .05

3. Studies on *Giardia microti*, by William C. Boeck. Pp. 85-136, plate 1, 19 figures in text ..... .60

4. A Comparison of the Life Cycle of *Crithidia* with that of *Trypanosoma* in the Invertebrate Host, by Irene McCulloch. Pp. 135-190, plates 2-6, 3 figures in text. October, 1919 ..... .60

5. A Muscid Larva of the San Francisco Bay Region which sucks the Blood of Nestling Birds, by O. E. Plath. Pp. 191-200. February, 1919 ..... .10

6. Binary Fission in *Collodictyon triciliatum* Carter, by Robert Clinton Rhodes. Pp. 201–274, plates 7–14, 4 figures in text. December, 1919 ..... 1.00

7. The Excretory System of a Stylet Cercaria, by William W. Cort. Pp. 275-281, 1 figure in text. August, 1919 ..... .10

8. A New Distome From Rana Aurora, by William W. Cort. Pp. 283–298, 5 figures in text. November, 1919..... .20

Vol. 20. 1. Studies on the Parasites of the Termites. I. On *Streblomastix strix*, a Polymastigote Flagellate with a Linear Plasmodial Phase, by Charles Atwood Kofoid and Olive Swezy. Pp. 1–20, plates 1–2, 1 figure in text. July, 1919 ..... .25

2. Studies on the Parasites of the Termites. II. On *Trichomitus termitidis*, a Polymastigote Flagellate with a Highly Developed Neuromotor System, by Charles Atwood Kofoid and Olive Swezy. Pp. 21–40, plates 3–4, 2 figures in text. July, 1919 ..... .25

3. Studies on the Parasites of the Termites. III. On *Trichonympha campanula* Sp. Nov., by Charles Atwood Kofoid and Olive Swezy. Pp. 41–98, plates 5–12, 4 figures in text. July, 1919 ..... .75

4. Studies on the Parasites of the Termites. IV. On *Leidyopsis sphaerica* Gen. Nov., Sp. Nov., by Charles Atwood Kofoid and Olive Swezy. Pp. 99–116, plates 13–14, 1 figure in text. July, 1919..... .25

Vol. 21. 1. A Revision of the *Microtus californicus* Group of Meadow Mice, by Remington Kellogg. Pp. 1-42, 1 figure in text. December, 1918 ..... .50

2. Five New Five-toed Kangaroo Rats from California, by Joseph Grinnell. Pp. 43-47. March, 1919 ..... .05